Reuse of agroindustry by-products to produce new fibre enriched bread formulations: impact in physico-chemical, sensorial and nutritional characteristics

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"Not all those who wonder are lost"
J.R.R. Tolkien, The Fellowship of the Ring

ABSTRACT

Bread is consumed in large quantities on a daily base and have an important role in human nutrition. The addition of functional ingredients to bakery products has risen in popularity due to the ability to reduce risk of chronic diseases beyond basic nutritional function. Food industrial by-products (BP) are rich sources of functional ingredients, such as fibre, minerals, and phytochemicals that are promising for improvement of nutritional quality of bread and may potentially enhance their health promoting properties. However, the incorporation BP functional ingredients also influence technological and sensorial properties.

The work developed in this PhD thesis aimed to obtain new fibre rich ingredients from agroindustry BP and to produce new types of fortified bread with enhanced nutritional/health benefits, while assuring good sensory characteristics. In order to achieve these goals, preliminary studies were carried out using brewer's spent yeast. Proteins/proteolytic enzymes from inner brewer's yeast had a negative impact on bread physical properties, whereas β-glucan rich extract can improve bread technological and health promoting properties. As result, β-glucans (fibre) rich extract was selected as a potential functional ingredient. Moreover, three agroindustry BP of plant origin were also used to obtain fibre rich extracts: elderberry skin, pulp and seeds (for elderberry extract, EE); orange peel (for orange extract, OE); pomegranate peel and interior membranes (for pomegranate extract, PE). Different extraction procedures were optimized for each BP in order to obtain fibre rich extracts that were characterized concerning their fibre composition. Functional breads eligible for nutrition claims "source of fibre" and "high in fibre" were prepared through wheat flour replacement with YE (4%), EE (4 and 36%), OE (4 and 8%), and PE (4% and 16%). The impact of wheat flour replacement was evaluated on: i) dough mechanical properties, microstructure and fermentation; ii) bread volume, texture, and image analysis; ii) sensory properties and consumer preference. Finally, the impact of bread fortification on total and bioaccessible mineral composition, estimated mineral daily intake, and the relationship between bioaccessibility and dietary fibre was evaluated. Results showed that: i) optimum water absorption increased with higher concentrations of OE, PE, and YE; ii) development time for EE, PE, and YE was shortened while the opposite was observed for OE; iii) the onset of starch gelatinization and maximum tan δ significantly increased with 36% EE and 4% PE; iv) protein structure, observed with confocal laser scanning microscopy, was modified at varying extents by the addition of extracts, and were in accordance with results from fundamental rheology; v) maximum and final dough height significantly decreased, except for 4% EE. Wheat flour replacement also had impact on bread parameters, since: i) volume and specific volume decreased at the highest concentrations in every extract; ii) significant changes were observed in crumb texture and structure, at higher extract concentrations. Multivariate PLS regression highlighted relationships between dough and bread data, and suggested that TDF content seems to have a stronger influence than its composition (SDF and IDF fractions). Breads suitable for "high in fibre" claim (8% OE, 16% PE, and 36% EE) were not viable, due to the detrimental results on dough and bread properties. Nonetheless, it was possible to successfully obtain breads with the nutrition claim "source of fibre" (replacement at 4%). Data obtained from external preference mapping pointed the selection of fibre rich extracts concentrations with best acceptance i.e., 7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE. At these concentrations, all bread formulations were eligible for the nutrition claim "source of fibre". Bread formulations with 7.0% EE and 5.0% PE registered more differences on colour and crumb structures than 2.5% OE and 2.5% YE, compared to control bread.

Data collected from image analysis complemented sensory profile information, whereas multivariate PLS regression provided information on the relationship between "Crust colour" and "Crumb colour" and instrumental data. Regression models developed for both sensory attributes presented good fitting (R2Y > 0.700) and predictive ability ($Q^2 >$ 0.500), with low RMSE. Crust and crumb a* parameters had a positive influence on "Crust colour" and "Crumb colour" models, while crust L* and b* had a negative influence. Fortification with 2.5% OE, 7.0% EE, and 5.0% PE improved the bread content on essential minerals when compared with control bread. The exception was bread fortified with 2.5% YE, which presented a mineral content similar to control bread, but its mineral bioaccessibility was significantly higher than in all the other bread formulations. The opposite was observed for 5.0% PE bread, which presented a significant reduction of bioaccessible minerals. Therefore the origin of fibre rich extract must be carefully selected, to avoid potential negative impact on mineral bioaccessibility. Overall, agroindustry by-products may improve bread nutritional/health properties, without major impact on dough and bread characteristics, and with sensory acceptability. Moreover, fibre rich extract obtained from brewer's spent yeast appears to have greater potential to be used in bread production.

Keywords: agroindustry by-products; functional ingredients; dietary fibre; dough properties; bread properties; mineral bioaccessibility

RESUMO

O pão é um alimento consumido diariamente, em grandes quantidades, e tem um papel importante na nutrição humana. A adição de ingredientes funcionais aos produtos de padaria tem atualmente elevada popularidade devido ao seu potencial de redução do risco de doenças crónicas, para além da função nutricional básica. Os subprodutos (BP) da indústria alimentar são fontes ricas em ingredientes funcionais, tais como, fibra, minerais e fitoquímicos, que são promissores para melhor a qualidade nutricional do pão e podem, potencialmente, melhorar suas propriedades de promoção da saúde. No entanto, a incorporação de ingredientes funcionais de BP também influencia as propriedades tecnológicas e sensoriais do pão.

O trabalho desenvolvido nesta tese de doutoramento visou a obtenção de novos ingredientes ricos em fibras a partir de BP da indústria agroalimentar, e a produção de novos tipos de pão fortificado que apresente benefícios nutricionais e de saúde, assegurando boas características sensoriais. Para atingir estes objetivos, foram realizados estudos preliminares utilizando levedura de cerveja. As proteínas/enzimas proteolíticas do interior da levedura de cerveja tiveram um impacto negativo nas propriedades físicas do pão, enquanto que o extrato rico em β-glucanos melhorou as propriedades tecnológicas e de promoção da saúde do pão. Como resultado, o extrato rico em β-glucanos (fibra) foi selecionado como um potencial ingrediente funcional. Adicionalmente, três BP da indústria agroalimentar de origem vegetal foram também utilizados para a obtenção de extratos ricos em fibras: pele, polpa e sementes de sabugueiro (para obter o extrato de sabugueiro, EE); casca de laranja (para obter o extrato de laranja, OE); casca e membranas interiores de romã (para obter o extrato de romã, PE). Diferentes procedimentos extrativos foram otimizados para cada BP, de forma a obter extratos ricos em fibra, que foram, posteriormente, caracterizados relativamente à composição em fibra.

Pães funcionais elegíveis para alegações nutricionais "fonte de fibra" e "rico em fibra" foram preparados através de substituição da farinha de trigo com YE (4%), EE (4 e 36%), OE (4 e 8%) e PE % E 16%). O impacto da substituição da farinha de trigo foi avaliado nas: i) propriedades mecânicas, microestrutura e fermentação da massa; ii) volume, textura e análise de imagem do pão; iii) propriedades sensoriais e preferência do consumidor. No final, foi avaliado o impacto da fortificação do pão na composição em minerais totais e bioacessíveis, bem como a ingestão diária estimada de minerais e a relação entre a bioacessibilidade e a fibra alimentar. Os resultados mostraram que: i) a absorção ótima de água aumentou com concentrações mais elevadas de OE, PE e YE; ii) o tempo de desenvolvimento da massa foi mais curto com EE, PE e YE enquanto o oposto foi observado com OE; iii) o início da gelatinização do amido e o tan δ máximo aumentaram significativamente com 36% de EE e 4% de PE; iv) a estrutura da proteína, observada com microscopia confocal de varredura a laser, foi alterada pela adição de extratos e estava de acordo com os resultados da reologia fundamental; v) a altura máxima e final da massa diminuiu significativamente, exceto com 4% EE. A substituição da farinha de trigo também teve impacto nos parâmetros do pão, uma vez que: i) o volume e o volume específico diminuíram nas concentrações mais elevadas de cada extrato; ii) foram observadas alterações significativas na textura e estrutura do miolo nas concentrações mais elevadas de cada extrato. A regressão de PLS multivariada destacou as relações entre os dados de massa e pão, sugerindo que o conteúdo de TDF parece ter uma influência mais forte do que sua composição (frações de SDF e IDF). Devido aos resultados negativos obtidos para as propriedades da massa e do pão, os pães elegíveis para alegação "rico em fibra" (8% de OE, 16% de PE e 36% de EE) não foram viáveis. No entanto, foi possível obter com sucesso pães com a alegação nutricional "fonte de fibra" (substituição a 4%). Os dados obtidos a partir do mapeamento de preferência externa permitiram a seleção das concentrações de extratos ricos em fibra com a melhor aceitação, isto é, 7,0% EE, 2,5% OE, 5,0% PE e 2,5% YE, sendo todas as formulações de pão elegíveis para a alegação nutricional "fonte de fibra". As formulações de pão com 7,0% de EE e 5,0% de PE registraram mais diferenças na cor e estrutura do miolo do que 2,5% de OE e 2,5% de YE, em comparação com o pão de controlo.

Os dados recolhidos da análise de imagem complementaram a informação sobre o perfil sensorial, enquanto a regressão de PLS multivariada forneceu informações sobre a relação entre "Cor de crosta" e "Cor do miolo" e os dados instrumentais. Os modelos de regressão desenvolvidos para estes atributos sensoriais apresentaram boa adequação ($R^2Y > 0,700$) e capacidade preditiva ($Q^2 > 0,500$), com baixo RMSE. Os parâmetros a* da crosta e do miolo tiveram uma influência positiva nos modelos "Cor de crosta" e "Cor do miolo", enquanto o L* e b* da crosta tiveram uma influência negativa. A fortificação do pão com 2,5% de OE, 7,0% de EE e 5,0% de PE melhorou o teor em minerais essenciais quando comparado com o pão controlo. O pão fortificado com 2,5% de YE foi a exceção, tendo apresentado um teor de minerais semelhante ao do pão controlo, mas com bioaccessibilidade significativamente superior à das restantes formulações de pão. O inverso foi observado para o pão com 5,0% PE, que apresentou uma redução significativa de minerais bioacessíveis. Portanto, a origem do extrato rico em fibra deve ser cuidadosamente selecionada, para evitar potenciais impactos negativos sobre a bioacessibilidade dos minerais. De uma forma geral, os subprodutos da indústria agroalimentar podem melhorar as propriedades nutricionais e de saúde do pão, sem grande impacto nas características da massa e do pão, e garantindo a aceitabilidade sensorial. Adicionalmente, o extrato rico em fibra obtido a partir de levedura de cerveja parece ser o que apresenta maior potencial para ser utilizado na produção de pão.

Palavras-chave: subprodutos da indústria agroalimentar; ingredientes funcionais; fibra alimentar; propriedades da massa; propriedades do pão; bioacessibilidade de minerais

LIST OF PUBLICATIONS

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*Presenting author

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Abbreviations	**Names**
1,3:1,4BG	(1-3)(1-4)-β-D-Glucan
1,3:1,6BG	(1-3)(1-6)-β-D-Glucan and (1-3)-β-D-Glucans
a*	Redness
AHC	Agglomerative hierarchical clustering
AI	Adequate intake
ANOVA	Analysis of variance
b*	Yellowness
BDL	Below limit of detection
BI	Bread with improver
BP	By-products
BPT	Bread fortified with proteolytic enzymes
BβG	Bread fortified with β-Glucans
C	Control bread
CA	Cell area
CAR–PDMS	Carboxen/Polydimethylsiloxane
CB	Control bread
CBA	Control breads from machine A
CBB	Control breads from machine B
DDT	Dough development time
DF	Dietary fibre
DMTA	Dynamic mechanical thermal analysis
DS	Dough stability
DWB	Dry weight basis
EBP	Elderberry skin, pulp and seeds
EDI	Estimated daily intake
EE	Elderberry extract
EFSA	European Food Safety Authority
FAAS	Flame atomic absorption spectrometry
FBA	Fortified breads with addition of 10 g of dry spent yeast from machine A
FBB	Fortified breads with addition of 10 g of dry spent yeast from machine B
FOV	Field of view

FW	Fresh weight
G^*	Maximum $\lvert G^* \rvert$ (Pa)
G^*_{onset}	$\lvert G^* \rvert$ at the starch gelatinization onset (Pa)
G_0^*	$\lvert G^* \rvert$ at 30 ºC (Pa)
GRAS	Generally recognized as safe
H,	Dough height at the end of measurement
H'_m	Maximum height of gaseous release
H_m	Maximum dough height
HS-SPME-GC/MS	Headspace–solid phase microextraction–gas chromatography/mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
IDF	Insoluble dietary fibre
L	Extensibility
L*	Lightness
LV	Latent variables
na	Not available
nd	Not detected
ns	Not significant
OAC	Oil absorption capacity
OBP	Orange peel
OE	Orange extract
OHC	Oil holding capacity
OWA	Optimum water absorption
P	Resistance to deformation or tenacity
P	Bread weight
P/L ratio	Elastic resistance and extensibility balance
PBP	Pomegranate peel and interior membranes
PCA	Principal component analysis
PE	Pomegranate extract
PLS	Partial least squares regression
Q^2	Cumulative predictive variation from internal cross-validation
R^2	Cumulative explained variation of Y explained in terms of sum of squares
R^2X	Cumulative explained variation of X explained in terms of sum of squares

R^2Y	Cumulative explained variation of Y explained in terms of sum of squares
RDA	Recommended dietary allowance
RMSE	Root mean square error
S	Weight of the displaced seeds
SDF	Soluble dietary fibre
SF	Degree of softening
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SIVD	Simulated *in vitro* digestion
SPME	Solid phase microextraction
SSF	Simulated salivary fluid
SV	Specific volume
SWC	Swelling capacity
T'_1	Time of H'_m
T_1	Time to reach H_m
tanδ	maximum tanδ
$\tan\delta_0$	tanδ at 30 ºC
TDF	Total dietary fibre
TG*	Temperature at maximum |G*| (ºC)
Ttanδ	Temperature at maximum tanδ (ºC)
T_x	Time of gas release
W	Deformation energy
WA	Water absorption
WHC	Water holding capacity
WHO	World Health Organization
YBP	spent yeas
YE	Yeast extract

GENERAL SCOPE AND OBJECTIVES

Bread is a basic dietary item dating back to the Neolithic era. Bread products vary widely around the world, as do their production techniques. Basic ingredients include cereal flour, water, yeast or another leavening agent, and salt (1). Generally, the bread making process is divided into three main steps: mixing, fermentation (proofing), and baking (1-4). Fermentation is caused by baker's yeast and brings the dough to optimum condition for baking. Dough expansion is determined by the stability of the gas cells and their ability to expand and retain gas (5). Every step in the processes need to be monitored, in order to guarantee the final product quality. Ingredients and processing conditions may have a strong impact in the final product quality and in consumer's acceptance.

The awareness of a healthy lifestyle is increasing; fewer calories, more fibre, less salt and safe additives are the consumer demands for a healthier bread Thus, food manufacturers have increasingly been focusing on added-value products as a way to improve profit margins and as a mean of differentiation. Consequently, breads containing functional ingredients became more important in the bakery industry and on the market. Therefore, new ingredients are necessary to develop those functional breads.

Agroindustry by-products (BP) differ on their origin and include a wide variety of constituents i.e., peel, stem, leaf, seed, shell, bran, kernel, pomace, oil cake, among others (6-12). Brewing wastes are another industry BP of great expression due to the high beer production. These BP can be good sources of dietary fibre (DF), which can be recovered and applied as bread ingredients. Additionally, these ingredients can be used to prepare functional breads with DF related nutrition claims i.e., "source of fibre" (3 g DF/100 g bread, fresh weight (fw)) and "high in fibre" (6 g DF/100 g bread, fw) (13). Therefore, incorporation of DF functional ingredients obtained from different by-products has impact not only on nutritional characteristics, but also on technological and health promoting properties of bread, as described in literature.

Fruit BP are not only interesting sources for DF extraction, but also as a source of antioxidants and minerals (14, 15). Orange BP (peel, pulp, rag, and seed) are mainly composed by dietary fibre and soluble sugars (16, 17). Pomegranate processing BP (peel and internal membranes) have high amounts of proteins and DF, and also minerals, polyphenols, and flavonoids (18-21). BP generated from elderberry processing (pomace) are a good source of anthocyanins and other polyphenols, vitamins, and DF (22, 23).

Brewing spent yeast, the second major BP from brewing industry can be rich source of functional ingredients, such as fibre (mainly β-glucans), vitamins, minerals, and protein including proteolytic enzymes (24-29).

Due to the global pressure towards sustainable environmental technology, alternative uses to agroindustry BP are paramount (2-6, 25, 30-32). However, processing of these raw materials meets challenges with respect to guarantee the stability of these ingredients and the physical, chemical and sensory quality of the resulting bread.

The main goals of this thesis were the production of new fibre rich ingredients obtained from agroindustry BP and its application on bread making to obtain new types of bread with enhanced nutritional/health benefits i.e., “source of fibre” and “high in fibre”, while keeping good sensory characteristics. For this purpose, fibre rich extracts (yeast extract, YE; elderberry extract, EE; orange extract, OE; pomegranate extract; PE) were obtained by different adjusted extraction procedures and characterized on DF content and profile. The impact of wheat flour replacement by the different fibre extract at various amounts was evaluated on dough, bread, and sensory analysis. In order to study the impact of bread enrichment on minerals bioaccessibility, simulated *in vitro* digestion was also evaluated. Figure i.1 shows a schematic summary of the major steps of this thesis.

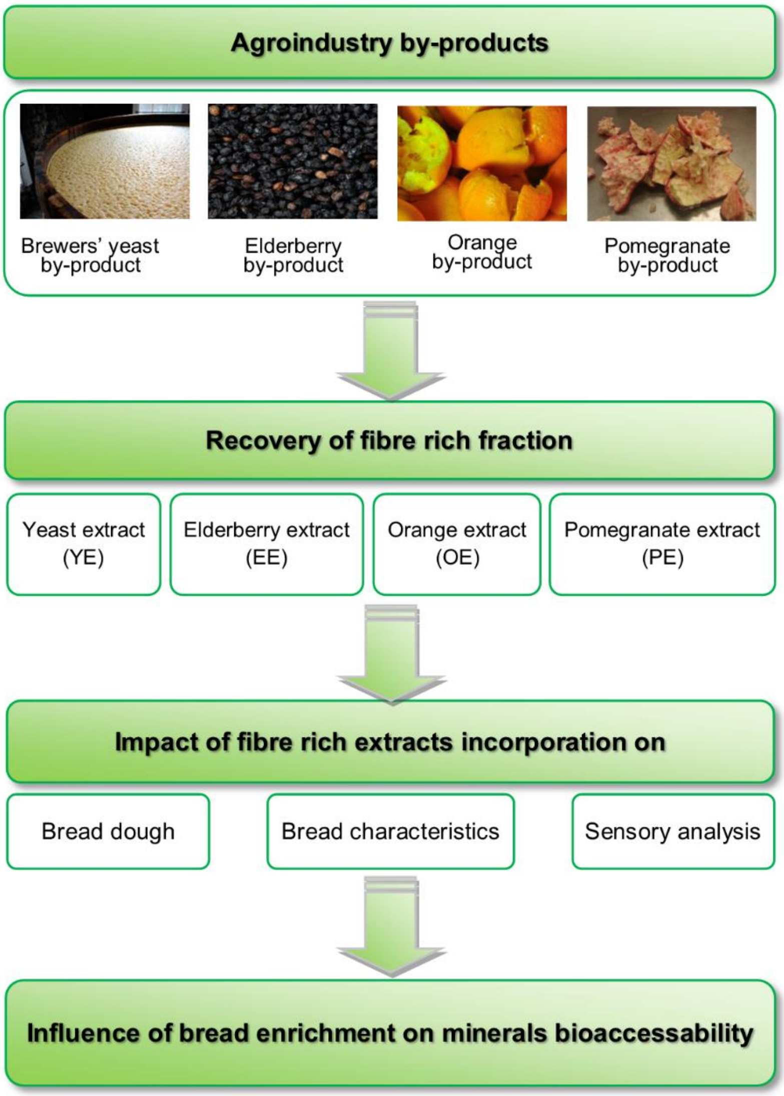

Figure i.1. Recovery of fibre rich extracts from agroindustry by-products for bread enrichment

To achieve the main goals of this thesis, different specific objectives were established:

1) Preliminary studies using brewer's yeast BP:
    a) Influence of homemade bread fortification with dry spent yeast, rich in β-glucans, on physical parameters, volatile composition and sensorial characteristics;
    b) Obtain β-glucan extract from brewer's spent yeast;
    c) Impact of bread fortification with β-glucan rich extract and proteolytic enzymes recovered from brewers' spent yeast on bread physical properties and quality;
2) Obtain fibre rich extracts from three agroindustry BP of plant origin: elderberry skin, pulp and seeds; orange peel; pomegranate peel and interior membranes;

3) Characterize DF composition of the four fibre rich extracts under study: YE, EE, OE, and PE;
4) Prepare functional breads presenting the nutrition claims “source of fibre” and “high in fibre”, through wheat flour replacement by the different fibre rich extracts and characterize those bread formulations concerning:
    a) Dough, including mechanical properties, microstructure and fermentation;
    b) Bread, including volume, texture, and image analysis;
    c) Correlations between fibre addition, dough and bread characteristics, through regression models.
5) Evaluate sensory, colour, and crumb structure properties of fibre enriched breads and application of a combined mathematical modelling approach to:
    a) Select fibre rich extracts concentrations with best acceptance by consumers;
    b) Understand the relationships between sensory and instrumental data;
6) Understand the impact of wheat bread fortification with fibre rich extracts on mineral composition, bioaccessibility, and estimated mineral daily intake.

THESIS OUTLINE

This thesis is divided in six parts and includes eight chapters. Chapters are closely related to each other, and the approach chosen in each one was dependent on the conclusions attained in the previous one(s). Each chapter contains its own introduction, materials and methods, results and discussion, and a brief conclusion. Figure i.2 shows a schematic overview of the organization of this thesis.

Part I includes **Chapter 1** and presents a comprehensive overview of food industry BP used as functional ingredients of wheat based bakery products. BP functional ingredients chemical and functional properties are described; also, their influence on nutritional profile of bakery products as well as technological and sensorial impacts is detailed.

Part II entitled "Brewer's yeast by-products impact on wheat bread characteristics" includes **Chapter 2 and 3**.

- **Chapter 2** describes bread fortification with dry spent yeast from brewing industry to increase β-glucans content and its impact on physical, chemical and sensorial characteristics of homemade bread.
- **Chapter 3** reports the impact of bread fortification with β-glucan rich extract and protein/proteolytic enzymes recovered from brewers' spent yeast on physical characteristics.

Part III entitled "Agroindustry by-products impact on wheat dough and bread characteristics" includes **Chapter 4 and 5**.

- **Chapter 4** describes fibre composition of EE, OE, PE, and YE and their impact on mechanical properties, microstructure and fermentation properties of dough, volume, texture, and image analysis of bread, and correlations between fibre addition, dough and bread characteristics through regression models.
- **Chapter 5** reports sensory, colour, and crumb structure properties of breads fortified with fibre rich fraction (OE, PE, EE, and YE) recovered from agroindustry BP, as well as statistical models developed for sensory preference evaluation and correlation with colour and crumb structure.

Part IV entitled “Agroindustry by-products influence on mineral bioaccessibility” includes **Chapter 6** and describes the impact of wheat bread fortification with fibre rich extracts on total and bioaccessible mineral composition of wheat breads, estimated mineral daily intake, and the relationship between bioaccessibility and dietary fibre.

Part V includes **Chapter 7** that presents the overall conclusions from this thesis as well as the future prospects.

Part VI includes **Chapter 8** with all the references cited throughout the thesis.

The experimental work was mainly developed in the following research laboratories:

- LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto (Portugal);
- Institute of Brewing and Beverage Technology, Research Group Cereal Technology and Process Engineering, Technical University of Munich (Germany).

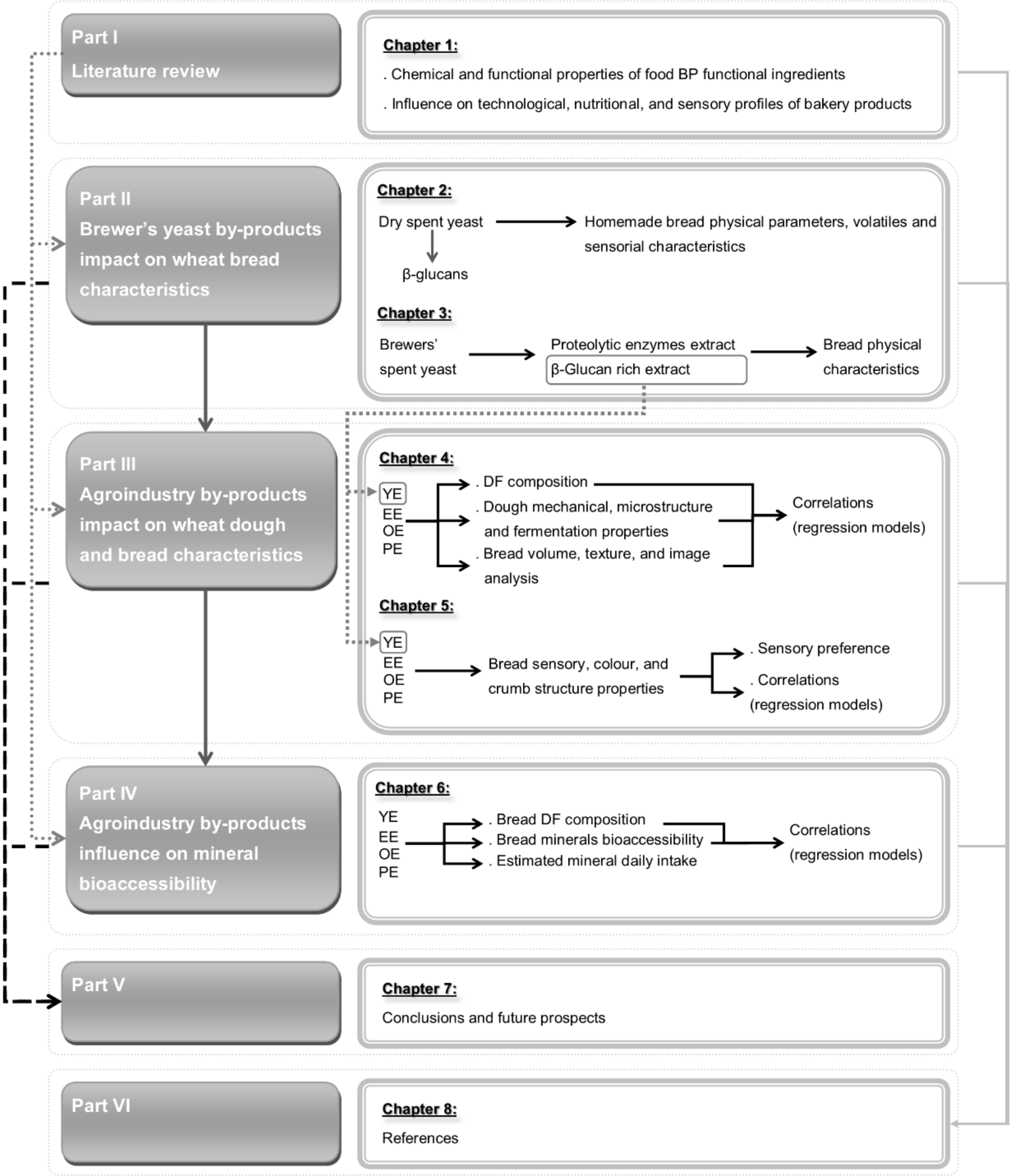

Figure i.2. Schematic overview of the thesis organization, with indication of the different chapters included in each part.

EE, Elderberry extract; OE, Orange extract; PE, Pomegranate extract; YE, Yeast extract.

PART I

CHAPTER 1

Literature review: Food industry by-products used as functional ingredients of bakery products

Abstract

Functional ingredients obtained from industrial by-products (BP) are a promising vehicle for the nutritional improvement of traditional bakery products and may enhance their health promoting properties. However, the incorporation BP functional ingredients also influence technological and sensorial properties. This review provides a comprehensive overview about the incorporation of potential functional ingredients obtained from a wide variety of food industrial by-products (Fruit and Vegetables; Cereals, Legumes, Nuts and Oilseeds; Brewery, Winery, and Distillery) to wheat based bakery products. Emphasis is given to the physico-chemical characterization of those ingredients, as well as their effect on dough and final products properties. Although physico-chemical composition of BP functional ingredients varies widely according to their source, it stands out by their content of total dietary fibre, protein, and/or minerals. BP functional ingredients incorporation on bakery products at 10% was frequently successful. Overall, BP functional ingredients improves nutritional profile of bakery products, however can also impair some functional and sensorial properties. From a nutritional point of view cakes and biscuits enriched with BP functional ingredients cannot compete with the healthier profile presented by breads

Keywords: Agroindustry by-products; bread; cakes, biscuits; functional ingredients

1.1. Introduction

Bakery products range in complexity and include items such as bread, cakes, biscuits (crackers and cookies), which contain wheat flour as main ingredient as it provides bulk and structure (33). Bakery products are consumed in large quantities on a daily base and have an important role in human nutrition. The addition of functional ingredients to bakery products has risen in popularity due to the ability to reduce risk of chronic diseases beyond basic nutritional functions (34). Food industrial by-products (BP) are rich sources of functional ingredients, such as fibre, minerals, and phytochemicals, among others (35-37). Finding alternative uses for those BP is of major interest. Nevertheless, they usually present, *per se*, insufficient biological stability, high water content, and high enzymatic activity. Thus, potential functional ingredients must be isolated or purified, before further use for the manufacture of bakery products.

Food industrial BP differ on their origin and include a wide range of components, namely: peel, stem, leaf, seed, shell, bran, kernel, pomace, oil cake, etc. (6-12). The incorporation of functional ingredients obtained from different BP has impact on technological, nutritional, and health promoting properties of bakery products, as described in literature. Available review articles on bakery products are focused on: health promoting characteristics (38-41), or specific BP fortification, namely wine pomace (42), brewer's spent grain (43), wheat bran (6, 44, 45), cereal bran (46), and fruit pomace (47). However, no review article gives a comprehensive overview on the addition of functional ingredients from food industrial BP added to bakery products, describing the nutritional, technological, and sensorial impacts.

This review focus on potential functional ingredients from industrial BP incorporated to wheat based bakery products. Physico-chemical characterization of those functional ingredients, and their effect on dough and final products properties will be summarized. Due to the wide variety of food industrial by-products existing in literature, they were organized according to their origin in three categories: Fruit and Vegetables; Cereals, Legumes, Nuts and Oilseeds; Brewery, Winery, and Distillery.

1.2. Composition of potential functional ingredients obtained from food industry

Fruit, vegetable, cereal, legume, nut, seed, brewery, winery, and distillery BP are rich sources of dietary fibre, proteins, vitamins, minerals and other bioactive compounds that can be recovered and used as value added functional ingredients (48). The composition of different food industrial BP that were incorporated in bakery products is summarized in Tables 1.1 to 1.3. It should be emphasized that for some BP, the authors did not indicate if data is expressed on fresh or dry weight basis. Thus, although data from those BP is still relevant, they were inserted in Tables 1.1 to 1.3, signalled with †, but were not used for the comparison comments described in this review.

1.2.1. Fruit and Vegetables

Fruit BP have been emphasized for their TDF content and well balanced proportion of insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), as well as for the presence of polyphenols, flavonoids, and carotenes (47). As described by Quiles *et al.* (47), BP resulting from juice extraction in apple, citrus, and plum have a high potential as TDF sources, with values of 35.5-89.8 g/100g dry weight basis (DWB), 35.4-68.3 g/100 g DWB, and up to 64.5 g/100 g DWB, respectively.

As shown in Table 1.1, TDF content in fruit peels and pulp can range from 11.2 to 75.27 g/100 g (DWB), where IDF accounts for 53 to 99% of the TDF. In general, the protein and fat content of these BP functional ingredients is below 10 and 5 g/100 g (DWB), respectively (49-53). Mineral content was also determined for banana peels by Eshak (50) and pomegranate peels by Ismail *et al.* (51). In both BP, K and Ca stand out, with levels higher than 1000 mg/kg (DWB). Major differences are observed for Mn, whose content in pomegranate peels represents 0.03% of the one found in banana peels. Pomaces from apple, date, and pear were analysed by Bchir *et al.* (54). In these pomaces, TDF is the main nutrient (apple pomace, 82.0 g/100 g DWB; date pomace, 83.7 g/100 g DWB; pear pomace, 90.7 g/100 g DWB), while protein concentration is below 11 g/100 g DWB and fat below 4 g/100 g DWB. Also, TDF is almost entirely composed of IDF (apple pomace, 95%; date pomace, 97%; pear pomace, 98%).

As expected vegetable BP composition differs according to the plant source (Table 1.1). Protein is the main nutrient in vegetable seeds, higher than 45 g/100 g DWB (55, 56). The major differences on fat content can be due to the use (or not) of a defatting step during BP recovery. Also, in pumpkin seed cake TDF is around 23.2 g/100 g DWB, with SDF accounting for 70% of TDF (55, 56). Regarding minerals, K stands out in fluted pumpkin seeds, with 13790 mg/kg DWB (55). The main nutrient obtained from sweet potato BP (leaves and stems), is TDF (higher than 40 g/100 g DWB). Differences are found in protein concentration, which are 4 times higher on leaves than on stems (57). For tomato pomace and celery spent residue, TDF is also the major nutrient (> 59 g/100 g DWB), while protein varies between 16.31 and 20.7 g/100 g DWB, and fat is below 7 g/100 g DWB (58-60). With respect to minerals, tomato pomace has higher concentrations of Mg and Ca, while Zn and Fe are higher in celery spent residue (58, 60).

Table 1.1. Composition of potential functional ingredients recovered from different BP from fruit and vegetables that were incorporated in bakery products (expressed in g/100 g, dry weight basis)

By-product source	Moisture	Protein	Fibre			Carbo-hydrates	Fat	Ash	Minerals[*]									Ref.
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Fruit and Vegetables																		
Fruit																		
Banana peel	6.39	8.74	11.2			46.93	4.54	22.2		3321	440	16303	641	54.73		1.97		(50)
Mango peel	7.49	1.56	59.44	38.63	20.81	73.57[a]	2.26	2.5										(52)
Mango peel	10.5	3.6	51.2	32.1	19.0	80.7[a]	2.5	3.4										(49)
Pineapple peel	6.28	5.74	75.27	74.16	1.1		1.61	3.18										(53)
Pomegranate peel	9.34	0.7	17.53			78.67	0.4	2.7		1192.04	592.94	2749.46		0.02	3.68	1.21	0.02	(51)
Mango pulp	10.04	2.87	47.68	33.75	13.93	76.49	1.24	1.29										(52)
Apple pomace	8.1	6	82	77.8	4.2		2.5	1.4										(54)
Date pomace	7.8	10.7	83.7	81.1	2.6		2.6	2										(54)
Pear pomace	8.3	5.7	90.7	89.2	1.5		3.7	0.8										(54)
Orange pulp[†]		9.79	74.87	54.81	20.06	9.27	2.43	2.66										(9)
Orange BP[†]	9.25	0.43	71.04	53.07	17.97		6.57	3.43										(61)
Mango kernel (defatted)[†]	7.801	10.046	3.773				1.042	2.568										(8)
Watermelon seed[†]	25.21-27.41	71.38-72.26						0.04-0.05										(37)
Watermelon seed[†]		79.1-83.8						0.2-0.3										(62)
Guava bagasse[†]	4.07	11.47	24.29				14.05	1.53										(63)

Table 1.1. Composition of potential functional ingredients recovered from different BP from fruit and vegetables that were incorporated in bakery products (expressed in g/100 g, dry weight basis) (continued)

By-product source	Moisture	Protein	Fibre			Carbo-hydrates	Fat	Ash	Minerals[*]									Ref.
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Fruit and Vegetables																		
Vegetables																		
Fluted pumpkin seed defatted)	6.7	49.2	2.3			44.3	0.9	6.3		749	205	13790	1489			45		(64)
Pumpkin seed cake	7.2	50.4	23.2	7	16.2	27	8.2	7.2										(56)
Sweet potato leaf	0.86-0.88	21.4-22.4	43.50-43.89			60.18-60.90[a]	5.57-7.36	11.2-11.3										(57)
Sweet potato stem	0.80-0.85	4.9-5.2	53.72-56.51			79.30-80.06[a]	3.52-4.96	9.77-11.98										(57)
Tomato pomace		16.31	59.94	55.03	4.91		5.38	3.492	2850.6	3625.5		24500.3	4625.1	40.1	41.5	130.5		(58)
Tomato pomace	4.71	20.7	76.27				5.2	4.75										(59)
Celery spent residue		19	61	53.5	7.5		5	7	626	2524	7104				62.5	288	20.5	(60)
Carrot pomace[†]	6.54	6.5	44.75	30	14.75			5.12										(65)
Tomato pomace[†]	10.2	18.25					0.65	7.25										(66)
Potato peel[†]		15.21-15.71	28.44-29.73	19.23-19.59	9.21-10.14	28.44-29.73[a]												(35)

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

[a] Total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

1.2.2. Cereals, Legumes, Nuts and Oilseeds

Wheat and rice bran have been extensively studied for their TDF content and potential source of natural bioactive compounds (6, 67). As described by Onipe *et al.* (6), these grain milling BP are composed by 33.4 to 63.0 g/100 g of TDF and 9.6 to 21.9 g/100 g of protein. These authors also reported some minerals in wheat bran, including Fe (19-340 mg/ kg), Zn (83-140 mg/kg), Mn (9-101 g/kg), Mg (5300-10300 mg/kg), and P (9000-15000 mg/kg) (6).

Bran composition varies widely, with values ranging from 6.59 to 21.8 g/100 g DWB for protein, 8.59 to 47.5 g/100 g DWB for TDF, and 1.14 to 20.7 g/100 g DWB for fat (68-71). Defatted rice bran, which is obtained from rice bran oil extraction, contains, in general, more nutrients than the original rice bran due to the concentration that occurs after fat extraction (38). Thus, protein content is almost 3 times higher in defatted rice bran than in rice bran, while TDF and fat is 16 to 18 times lower, respectively (68, 69, 71, 72). Millet seed coat is another example of cereal BP, with protein, TDF, and fat contents similar to some cereal brans (Table 1.2) (68, 70, 71, 73). Ca and P are the minerals present at higher concentrations in millet seed coat (73). Sugarcane bagasse has higher TDF content and IDF fraction than cereal BP (TDF is 2 to 9 times higher than in cereal BP and IDF accounts for 99% of TDF), while protein and fat levels are lower (68-71, 73, 74).

Some BP resulting from legume processing includes pods, brokens, powder, husks, and cakes, as shown in Table 1.2. As reported by Belghith Fendri *et al.* (75), some differences are found between broad bean and pea pods for TDF and fat, while protein values are similar. TDF is the main component for both pods and IDF represents 94 % to 95% of existing TDF. Nevertheless, TDF content is 1.2 times higher and fat is 4 times lower for broad bean pods, compared to pea pods. Minerals are also described for both pods and higher concentrations of K, Mn, and Zn are found for broad bean pod presenting, while pea pods have higher levels of Mg, Ca, Na, and Cu (75). For pigeon pea brokens, powder, and husks protein and TDF concentration differs from what is found for chickpea, lentil, and pea hulls. Protein content is 2.4 to 5.7 times higher for pigeon pea brokens, powder, and husks, whereas TDF is 16 to 19 times higher for hulls (76, 77). Baru, groundnut, and soybean cakes are BP resulting from legume oil extraction. These cakes have similar TDF content (38.8-40.2 g/100 g DWB), which is primarily comprised of IDF fraction (86-92%), but vary for protein (29.46-50.5 g/100 g DWB) and fat (2.1-11.84 g/100 g DWB) (78, 79). Nevertheless, cakes stand out from other legume BP with 1 to 9.7 times higher protein concentration (75-79). Minerals, such as Ca, Na, K, Zn, Fe, and Cu can as well be found in baru cake (79).

With respect to nuts and oil seeds, peels resulting from cupuassu processing are predominantly comprised of TDF (79.81 g/100 g DWB), where 98% corresponds to IDF fraction (80). Protein and fat levels in cupuassu peels are lower than 3 g/100 g DWB (Salgado et al., 2011). As shown in Table 1.2, oilseeds cakes composition varies at some extent (56). Comparing with other cakes, sunflower seed cake has higher protein content (1.3 to 1.4 times), and lower values for TDF (1.3 to 1.4 times) and fat (1.6 times) (56). Unlike other BP, SDF is the major TDF fraction for oilseed cakes, accounting for 54 to 83% of TDF (56).

Table 1.2. Composition of potential functional ingredients recovered from different BP from cereals, legumes, nuts and oilseeds that were incorporated in bakery products (expressed in g/100 g, dry weight basis)

By-product source	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals***									**Ref.**
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Cereals, Legumes, Nuts and Oilseeds																		
Cereals																		
Wheat bran		11.67-15.52	8.59-28.87	4.63-23.64	2.65-5.23			0.91-2.27										(70)
Wheat bran		19.1-21.8	40.8-47.5					5.9-6.5										(69)
Wheat bran	9.28	17.11					4.82	5.28										(68)
Rice bran	7.99	14.54					16.64	9.2										(68)
Rice bran	0.8	12.9	29.4	28.4	1.1	26.7	20.7	10.3										(71)
Corn bran	9.55	6.59					1.14	0.85										(68)
Oat bran	9.23	12.19					6.28	3.87										(68)
Rice bran (defatted)	5.6	39.8	1.8			49.9	1.5	6.9										(72)
Millet seed coat	11	9.5-13.4	39.6-48.8	38.4-47.7	1.1-1.4	16.5-18.8	2.6-3.7	4.3-5.1		7070-8640			2530-3690		220-270	550-750		(73)
Sugarcane bagasse	3.24-4.5	0.83-0.96	72.92-76.60	72.47-76.01	0.40-0.59		0.01-0.30	0.74-1.59										(74)
Rice bran †	18.00-18.17	12.60-13.25					16.67-16.93		253.89-270.12	235.21-244.88				15.83-16.53	22.06-24.44	60.19-63.24		(81)
Rice bran (defatted) †	13.3	16.6	7.41	5.17	2.24		0.33	15.6		240			160			285		(82)
Rice bran (defatted) †	7.6	20.5	11.6				1.0	11.5	9530	5300	75	12800						(83)
Rice bran (defatted)†	10.31-10.98	2.69-8.35	62.73-82.94				0.59-2.88	3.17-4.21										(84)
Barley rootlets†		36.75	43.01	39.21	3.8	60	1.7		0.8	0.6	0.1	21.7	5.2		0.1			(85)

Table 1.2. Composition of potential functional ingredients recovered from different BP from cereals, legumes, nuts and oilseeds that were incorporated in bakery products (expressed in g/100 g, dry weight basis) (continued)

By-product source	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals***									**Ref.**
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Cereals, Legumes, Nuts and Oilseeds																		
Legumes																		
Broad bean pod		13.46	53.01	34.69	18.32	17.26	0.24	9.02	2.75	3.24	4.77	20.13		24.76	19.84		7.65	(75)
Pea pod		13.37	43.87	35.61	8.27	24.34	1.06	8.07	5.07	30.43	17.39	4.09		9.23	9.23		10.86	(75)
Pigeon pea brokens, powder and husk		29.42	4.66			54.88	5.73	5.32										(77)
Chickpea hull		12.1	74.8					5.7										(76)
Lentil hull		9.7	86.7					2.3										(76)
Pea hull		5.2	88.9					3.3										(76)
Baru cake (partially defatted)	3.63	29.46	38.8	33.73	5.07	11.57	11.84	4.7		2009.1	95.5	12176			76.2	132.9	20.4	(79)
Groundnut cake		42.7	39.4	36.2	3.2		9.0	6.72										(78)
Soybean cake		50.5	40.2	34.6	5.6		2.1	7.48										(78)
Broad bean pod†	4.3	6.6	91.61	86.38	5.23		0.66	3.71										(75)
Pea pod†	4.5	6.73	89.86	85.5	4.36		0.87	3.56										(75)
Nuts and Oilseeds																		
Cupuassu peel	1.45	2.84	79.81	78.29	1.52		1.91	2.45										(80)
Sunflower seed cake	7.6	52.5	26.9	4.5	22.4	30.1	9.8	7.6										(56)
Walnut seed cake	3.6	40.9	38.0			38.0	16.1	5.1										(56)
Yellow linseed cake	7.4	37.3	35.0	16.0	19.0	35.0	15.4	5.8										(56)

Table 1.2. Composition of potential functional ingredients recovered from different BP from cereals, legumes, nuts and oilseeds that were incorporated in bakery products (expressed in g/100 g, dry weight basis) (continued)

By-product source	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals**[*]									**Ref.**
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Cereals, Legumes, Nuts and Oilseeds																		
Nuts and Oilseeds																		
Cashew apple bagasse[†]	6.52	7.63	3.26				3.7	1.42										(63)

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

[a] Total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

1.2.3. Brewery, Distillery, and Winery

Nutrient content in brewery and distillery spent grains, as well as in grape pomace have been described to vary according to the nature of the raw material, e.g. barley, wheat, or corn (42, 43, 86). Data on composition of brewery, distillery, and winery BP used for incorporation in wheat bakery products is scarce (Table 1.3), however they have been extensively characterized for other purposes. According to Lynch *et al.* (43), brewer's spent grain is mostly composed by TDF (59.1-74.1 g/100 g DWB), including hemicellulose, cellulose, and lignin. Protein ranges from 14.2 to 31.0 g/100 g DWB and fat from 3.0 to 13.0 g/100 g DWB (43). As reported by Liu (86), protein is main nutrient in distiller's grain, which varies from 23.4 to 38.9 g/100 g DWB. Fat concentration in distiller's grain is similar to what is described for brewer's spent grain (3.6-12.8 g/100 g DWB), whereas TDF is lower (5.6-10.9 g/100g DWB). Mineral composition is also documented for distiller's grain by Liu (86): Ca (280-2900 mg/kg), P (6800-8900 mg/kg), K (9100-11440 mg/kg), Mg (2800-3450 mg/kg), sulphur (S) (4700-8400 mg/kg), Na (1300-2630 mg/kg), Zn (6.1-113.7 mg/kg), Mn (15.8-22.0 mg/kg), Cu (5.55-10.00 mg/kg), and Fe (21.47-149.00 mg/kg). According to García-Lomillo and González-SanJosé (42), TDF is the main component in grape pomace (43-75 g/100 g DWB), including cell wall polysaccharides and lignin. Protein concentration ranges from 6 to 15 g/100 g DWB and fat from 14 to 17 g/100 g DWB. García-Lomillo and González-SanJosé (42) also describe some variations on mineral content between skins and seeds: skins present higher levels of K, where Ca, P, S, and Mg are higher in seeds.

Briefly, pomaces from fruit, vegetable, and winery, present some differences in TDF content that is higher for fruit pomaces (82-90.7 g/100 g DWB *versus* 59.94-76.27 g/100 g DWB in vegetables and winery pomaces). Pomaces present variable protein and fat concentration (11.49-20.7 g/100g for protein and 2.81-5.38 g/100 g for fat) (54, 58, 59, 65, 66, 87-89). Vegetable seeds have higher protein levels (49.2 g/100 g) when compared with winery seeds (17.4 g/100 g) (55, 90). Regarding cakes from legumes and nuts, major differences are found for protein (29.46-52.5 g/100 g DWB), TDF (26.9-40.2 g/100 g DWB), and fat (2.1-16.1 g/100 g DWB) (56, 78, 79).

Table 1.3. Composition of potential functional ingredients recovered from different BP from brewery, winery, and distillery that were incorporated in bakery products (expressed in g/100 g, dry weight basis)

By-product source	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals**[*]									**Ref.**
			TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Brewery, Winery, and Distillery																		
Brewery																		
Brewer's spent grain[†]		22.13				64.88	7.12		2.4	2.2	0.1	0.7	4.6		0.1	0.1		(91)
Brewer's spent grain[†]	6.14	23.19	12.85			51.39	2.79	16.98										(92)
Distillery																		
Distiller's grain	12.85	6.78					0.43	5.36										(93)
Winery																		
Grape seed	7.4	17.4						4.9										(90)
Grape pomace skins		11.49	67.95			1.4	2.81	10.53										(87)

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

[a] Total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

1.3. Functional properties of ingredients from food industry BP

Functional properties are intrinsic physicochemical characteristics and are usually linked to the interaction between water and oil. They include, water holding capacity (WHC), oil absorption capacity (OAC), oil holding capacity (OHC) and swelling capacity (SWC). Functional properties can be used to foresee the technological impact of a given ingredient on a food product. For instance, higher OHC is preferable for stabilization of food products with high fat content, and emulsions, whereas high WHC helps to avoid syneresis and alters viscosity and texture in some food products (94). Functional properties of different food industry BP grouped according to their origin are summarized in Table 1.4.

In fruits, WHC and SWC are higher in peels than in pomace (54, 95). For orange BP, WHC values are in the same range of pomace and SWC was comparable to peels (61). Kuchtová *et al.* (89), suggested that high WHC and SWC depend on SDF content, while the high protein and fat concentrations can impair hydration capacity (WHC and SWC). SDF fraction found for apple peels by Jun *et al.* (95) is higher (22% of TDF) than what is reported by Bchir *et al.* (54) for apple, date, and pear pomaces (4, 3, and 2%, respectively). These differences in SDF fraction can therefore explain higher WHC and SWC for apple peels. OHC is lower in orange BP than for pomaces (Table 1.4) which could be related to lower protein concentration (0.43 g/100 g in orange BP versus 6.0, 10.7, and 5.7 g/100 g DWB for apple, date, and pear pomaces) (50, 54, 61).

In vegetables, higher WHC and SWC are observed for pumpkin pomace than for potato peels (35, 89). No comparison can be establish on SDF content, as it was not determined for pumpkin pomace. It has been suggested by Kaur and Singh (96) that higher OAC could be related with the presence of hydrophobic proteins that exhibit superior fat binding. OAC was similar in both BP, which also present comparable results for protein concentration (15.21-15.71 g/100 g DWB in potato peels and 12.35 g/100 g in pumpkin pomace) (35, 89).

Functional properties of cereals and legumes BP are also summarized in Table 1.4. In cereals, WHC, SWC, and OAC can be higher in rice straw than in brans or sugar bagasse (72, 74, 82, 84, 97, 98). As data on chemical composition is missing in some BP (not determined by authors), it is not possible to establish a relationship between SDF content and WHC, SWC, and OAC values.

In legumes, higher WHC and lower OHC are observed for broad bean pod than for pea pod (36, 75). The higher SDF proportion found in broad bean pod (6 and 35% of TDF *versus* 5 and 19% for pea pod) could explain higher WHC values. Although broad bean present lower OHC than pea pod, no major differences are observed on protein content.

Table 1.4. Functional properties of ingredients obtained from different BP that were incorporated in bakery products.

By-product source	WHC (g water/g)	OAC (g oil/g)	OHC (g oil/g)	SWC (cm^3/g)	Ref.
Fruit and Vegetables					
Fruit					
Apple peel	9.96	6.28		13.43	(95)
Apple pomace	7.5		2.2	7	(54)
Date pomace	5.7		2.3	3.9	(54)
Pear pomace	4.9		2.1	5.9	(54)
Orange BP	5.08-6.44		1.23-1.42	11.79-11.98	(61)
Vegetables					
Potato peel	3.37-4.45	2.07-2.08		2.58-4.68	(35)
Pumpkin pomace	5.70	2.63		10.26	(89)
Cereals, Legumes, Nuts and Oilseeds					
Cereals					
Rice straw	7.35-12.17*	4.00-11.74		4.56-10.62	(98)
Oat bran	5.99				(99)
Rice bran (defatted)			2.3*		(72)
Rice bran (defatted)	5.11-5.20*	4.35-4.96*		5.93-6.08	(84)
Rice bran (defatted)	2.1*		1.8*		(82)
Sugarcane bagasse	3.7			7.1	(74)
Legumes					
Broad bean pod	6.98		3.39		(75)
Broad bean pod	4.46		1.415		(75)
Pea pod	4.64		2.86		(75)
Pea pod	3.69		1.14		(75)
Brewery, Winery, and Distillery					
Distillery					
Distiller's grain	0.541				(93)

* ml/g

OAC, Oil absorption capacity; OHC, Oil holding capacity; SWC, swelling capacity; WHC, Water holding capacity.

1.4. Wheat bakery products with ingredients obtained from food BP

Wheat (*Triticum aestivum*) is a major agricultural product and an essential crop for bakery products due to its baking performance that outstands other cereals (100). The incorporation of potential functional ingredients from BP on wheat bakery products can improve their nutritional value and health promoting properties. However, they can also impair some functional and sensory properties. A balance in incorporation of functional ingredients from BP has to be found, in order to obtain desirable bakery products that can compete with the traditional ones.

1.4.1. Wheat Bread

Wheat flour, yeast, and salt are the base ingredients for bread. Bread can be classified as either leavened or unleavened. In leavened bread, dough contains leavening agents (baker's yeast or baking powders) that produce carbon dioxide, responsible for bread aeration. In this type of bread, rigorous control of yeast behaviour throughout fermentation and proofing are required. In unleavened bread, there is no carbon dioxide production and resulting bread is frequently flat and dense (1, 101). Bread making includes a series of processes, namely, mixing, fermentation, proofing, baking, and cooling. Every step in the processes needs to be monitored, in order to guarantee the quality of final product. Additionally, different ingredients and additives can be used in order to obtain special types of bread. The application of food industry by-products as functional ingredients of bread opens new possibilities.

1.4.1.1. Wheat bread enrichment with new ingredients from food industry BP

Nutritional composition of breads with incorporation of potential functional ingredients from different BP is shown on Table 1.5. As indicated in the Table 1.5 in some cases authors do not indicate if data is expressed in fresh or dry weight basis. Only data presented in fresh weight is used for comparisons described in the text. When compared with the respective control breads (data presented on bold within brackets), BP incorporation increased protein levels from 3 to 106% (50, 80, 82, 84, 102-105). An exception was observed in the study conducted by Soares Júnior *et al.* (106), where protein content decreased from 5 to 15%, compared to the control. Bread TDF values rise from 5 to 458% with BP addition, compared with the respective control. Moreover, higher TDF values are found for bread with cupuassu peel, pomegranate bagasse and rice bran (80, 104, 106). TDF values can also be used to establish the eligibility of a given bread formulation for the nutrition claims "source of fibre" (≥ 3 g fibre/100 g food product) and "high in fibre" (≥ 6 g fibre/100 g food product) (107). Control breads used in studies carried out by Bhol *et al.* (104) for pomegranate bagasse incorporation and by Soares Júnior *et al.* (106) for rice bran incorporation are already a "source of fibre". In these studies, TDF content increase in bread formulations with 15% pomegranate bagasse, and 30% rice bran, this makes them "high in fibre". Unlike their control breads, formulations with 20% of brewer's spent grain, 3 and 6% cupuassu peel, are a "source of fibre" , while breads with 9% cupuassu peel, 15% pomegranate bagasse, and 30% rice bran are "high in fibre" (80, 105). Fat content in wheat bread formulations increases from 2 to 149% with BP addition, compared with the controls (50, 80, 102, 104-106). In addition, fat content is the highest in wheat breads formulations with Cupuassu peel, rice bran, and respective controls (50, 80, 82, 84, 102-106). Fat content can also be used to establish the eligibility for the fat related nutrition claims "low fat" (< 3 g fat/100 g food product) and "fat-free" (< 0.5 g fat/100 g food product) (107). As observed in Table 1.5, control breads of banana peels, pomegranate bagasse, and rice bran are "low fat", while brewer's spent grain bread control is "fat-free" (50, 102, 104-106). As BP addition increases fat levels, the eligibility to fat related nutrition claims is lost. Nevertheless, wheat breads with banana peels, 10, 15, and 20% brewer's spent grain, 5% pomegranate bagasse, and 5 and 10% rice bran are still "low fat", while 5% brewer's spent grain is "fat-free" (50, 102, 104-106). Regarding minerals, incorporation of banana peels, pomegranate bagasse, and rice bran generally increases mineral content (50, 102, 104). However, some exceptions are observed i.e., banana peels decrease P content, pomegranate bagasse slightly reduces Mg, K, and Zn levels, and Na concentration are lower with rice bran addition (50, 102, 104). Mineral related nutrition claims include "source of [name of mineral]" (≥ 15% of corresponding RDA value/100 g food product), "high in [name of mineral]" (≥ 30% of corresponding RDA value/100 g food product), "low Na" (< 0.04 g Na/100 g food product), and "Na free" (< 0.005 g Na/100 g food product) (107). Control bread used in the study of banana peels incorporation by Eshak (50) is already a "source of P", "source in Fe", and "high in Mn". Although banana peels addition decrease P content, all formulations are still a "source of P". Also, formulations with 5 and 10% addition of banana peels are "high in Fe", while 10% addition is "high in K" (50). All bread formulations (including control) analysed by Bhol *et al.* (104) are "Na free". Additionally, while control bread is a "source of Cu", bread with 5 and 15% addition of pomegranate bagasse are "high in Cu" (104). Regarding mineral content of wheat breads studied by Soares Júnior *et al.* (106) control bread is already a "source of Fe", whereas formulations with addition of rice bran (5, 10, and 15%) are "high in Fe". Bread formulation at 10 % addition is a "source of Mg" while at 15% addition is a "source of Ca" (106).

Modifications on enriched wheat breads dough rheology are summarized in Table 1.6. Rheology has an important role in cereal industry, especially in what concerns breadmaking (40). Rheological properties of food materials can be measured or tested with different instruments (farinograph, alveograph, mixolab, visco anlyser), which provide data that can be correlated with final product's quality.

Dough water absorption (WA), a mixing property of dough, increases with BP addition to wheat bread (56, 57, 59, 61, 82, 103, 108, 109). The only exception is registered for rice bran addition studied by Anil (68), where WA decreases. WA is negatively correlated with protein content, but usually increases with TDF (110-112). Water absorption increase could be due to TDF, through the great number of hydroxyl groups existing in the fibre structure, which allow more water interactions through hydrogen bonding (111). Water absorption decrease observed by Anil (68) for rice bran enriched wheat dough could, in turn, be related to the TDF chemical structure, association between molecules, particles size, and fibre porosity (113). Moreover, hydration properties, such as WHC, of BP incorporated in dough would also be expected to influence WA. However, as mentioned by Ktenioudaki and Gallagher (40), this relationship has not been established yet, probably because hydration properties are mostly determined by BP itself whereas gluten present in the dough is the main influence for WA.

The influence of BP on other mixing properties of wheat bread dough, such as development time (DDT), dough stability (DS), and degree of softening (SF), varied according with BP and concentrations added (56, 57, 59, 61, 68, 82, 103, 108, 109). Changes in DDT and DS can be related to TDF composition of BP. The addition of these BP to wheat flour increases the level of TDF and changes dough properties. On one hand, an increase in dough consistence can occur, due to the dilution of wheat protein, or presence of hydrophilic components in BP that may quickly absorb water, and consequently reduce DDT (114, 115). DDT can in turn increase if TDF present in BP absorbs water slowly (56). On the other hand, an increase in TDF can lead to a larger number of hydroxyl groups available for interaction with water through hydrogen bonding during dough development (116). Although being individually weak, when large numbers of hydrogen bonds are established they provide stability to the dough (increased DS) (41). Gluten dilution can also be responsible for a decrease in DS, as it weakens protein crosslink and reduces chains interactions, influencing the formation and expansion of the gluten network (56, 61). Modifications on degree of softening (SF) or mixing tolerance index depend on the tolerance towards mixing. Dough with lower SF values is preferred, while higher values often indicate more difficulties during mechanical handling and makeup of the dough.

As seen in Table 1.6, BP incorporation in wheat bread often resulted in a decrease on volume and specific volume (36, 53, 56, 57, 61, 82, 84, 85, 98, 104, 106, 117-121). Pomeranz *et al.* (122) suggested that this reduction in volume and specific volume occurs by dilution of the gluten content and changes in crumb structure, which in turns impairs carbon dioxide retention. Also, Chen *et al.* (123) suggested that TDF molecules may perhaps interact with wheat flour proteins and interfere with gluten development.

Regarding texture of wheat breads with incorporation of potential functional ingredients from different BP (Table 1.6), an overall increase is observed for hardness/firmness and chewiness (except pea pod and tomato pomace), and a decrease for cohesiveness (except for mango peel) (36, 53, 84, 85, 98, 115, 117-119, 121, 124-126). As for springiness, variations were found between different formulations (53, 98, 117, 119, 124, 126, 127). Hardness is mostly attributed to the amylose and amylopectin matrix, which contribute to overall bread texture (128, 129). Moreover, as described by Gómez *et al.* (130), bread hardness can also result from interactions between gluten and TDF. Cohesiveness, springiness, and chewiness (product of both cohesiveness and springiness) are influenced by interactions between gelatinized starch and gluten dough that can create elastic dough and form bread sponge structure after heating (129, 131).

A decrease in wheat bread L* is frequently observed with the addition of potential functional ingredients from different BP (Table 1.6), except for brewer’s spent yeast (36, 43, 53, 61, 68, 71, 85, 98, 117, 119, 121, 126, 127, 132, 133). A darker colour usually results from Maillard and caramelization reactions, but the original colour of BP can also influence (134, 135). Variations on a* and b* values could likely be due to the original BP colour.

As regards to sensory analysis, scores related with aroma, flavour, taste, and texture generally decrease with the increase of BP addition (Table 1.6) (36, 53, 57, 61, 68, 71, 80, 82, 85, 98, 103-106, 108, 109, 118-121, 126, 132, 133). However, in some wheat bread formulations this effect is not observed and BP addition has either no influence on bread quality (brewer’s spent grain and grape seed) or even improves the scores of some attributes (brewer’s spent yeast, pomegranate bagasse, pumpkin seed cake, sunflower seed cake, tomato pomace, walnut seed cake, and yellow linseed cake) (56, 59, 90, 91, 117, 127).

Taking into consideration all analysed parameters, especially sensory analysis, highest BP addition to wheat breads without overall significant differences is often 10%. The maximum added amount described is 20% (oat bran and wheat bran), while the minimum is 2% (orange BP) (53, 56, 61, 68, 82, 85, 91, 105, 108, 109, 121, 125-127).

Table 1.5. Nutritional composition of bread with incorporation of potential functional ingredients obtained from different BP (expressed in g/100 g, fresh weight basis)

By-product source	% added	Moisture	Protein	Fibre			Carbo-hydrates	Fat	Ash	Minerals*									Ref.
				TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Bread																			
Banana peels	5 and 10	1.61-2.59 **(0.65)**	11.60-12.20 **(10.72)**	1.94-2.12 **(1.41)**			43.19-49.69 **(74.44)**	2.20-2.88 **(2.05)**	32.96-37.07 **(10.73)**		663-915 **(229)**	5659-6066 **(5142)**	5353-8194 **(873)**	1086-1180 **(1232)**	11.19-15.18 **(6.16)**	3.2-5.2 **(0.15)**	96-221 **(37.71)**		(50)
Cupuassu peel	3, 6, and 9	25.28-28.78 **(26.08)**	10.15-10.41 **(10.15)**	4.54-7.15 **(2.32)**	3.14-5.82 **(1.10)**	1.31-1.40 **(1.22)**	46.70-53.33 **(55.02)**	5.14-5.29 **(5.03)**	1.61-1.66 **(1.45)**										(80)
Pomegranat bagasse	5 and 15	36.35-37.92 **(35.15)**	8.03-8.27 **(7.8)**	4.8-6.8 **(3.2)**			44.03-46.21 **(51.00)**	2.37-3.74 **(2.20)**	0.67-0.81 **(0.65)**	1.11-1.14 **(1.52)**	39.2-40.8 **(5.10)**	14.8-15.8 **(14.7)**	22.79-23.61 **(24.56)**	8.7-9.1 **(9.9)**		0.72-0.80 **(0.80)**	2.2-2.7 **(1.2)**	3.5-3.6 **(2.1)**	(104)
Brewer's spent grain	5, 10, 15, and 20	38.51-42.09 **(37.43)**	7.49-10.03 **(6.64)**	1.91-4.52 **(0.81)**			40.46-49.16 **(53.69)**	0.48-0.97 **(0.39)**	0.68-1.29 **(0.44)**										(105)
Rice bran	7.5, 15, 22.5, and 30	32.29-37.28 **(29.84)**	6.90-7.69 **(8.14)**	5.23-7.20 **(4.15)**	3.56-5.00 **(2.64)**	1.21-1.68 **(1.51)**	38.89-50.00 **(54.56)**	3.61-5.59 **(2.41)**	1.38-3.84 **(0.90)**										(106)
Rice bran	5, 10, and 15	21.78-23.67 **(21.07)**	11.54-12.01 **(11.04)**	1.84-2.91 **(1.76)**			58.11-61.00 **(63.10)**	2.06-3.41 **(1.57)**	1.78-2.41 **(1.46)**	482.8-1322.2 **(136.5)**	985.4-1307.0 **(813.1)**	2530.3-2858.4 **(3052.5)**	1104.2-1882.0 **(807.4)**				114.0-205.2 **(93.2)**		(102)
Rice bran (defatted) †	1, 2, 3, 4, and 6			2.53-7.98 **(1.87)**															(84)
Rice bran (defatted) †	5, 10, and 15			8.42-10.91 **(6.31)**	7.05-9.25 **(5.25)**	1.37-1.66 **(1.06)**													(82)
Soy hull†	1.5, 3.0, 4.5, 6.0, and 7.5	7.83-7.95 **(7.98)**	12.95-13.27 **(11.85)**	2.63-3.99 **(2.11)**				1.98-2.29 **(1.91)**	1.95-2.10 **(1.92)**	1836.5-2818.4 **(1530.0)**	602.5-1087.5 **(465.0)**				38.8-43.0 **(35.3)**	38.4-45.8 **(34.0)**	50.3-62.1 **(44.0)**	8.6-16.0 **(6.3)**	(103)

Values in bold within brackets are relative to control bakery product used in each study.

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

‡ Values by dry weight basis, but no information on moisture.

[a] Total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

Table 1.6. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of bread

By-product source	% added	Dough properties	Bread properties			Sensory scores (highest concentration)	Higher addition[1]	Ref.
		Rheology	Physical characteristics	Texture	Colour			
Orange peel	2.5 and 5.0		↓ specific volume		↑ b*	↓ crumb colour, aroma, taste, texture, and overall acceptability	2.5%	(120)
Mango peel	1, 3, and 5	↑ G', G'', and η*; ↓ tan δ	↑ moisture and density; ↓ specific volume, weight loss and loaf height	↑ hardness, cohesiveness and springiness	↑ brownness index; ↓ whiteness index	↑ fruity aroma, fruity taste, after taste, crumb colour, hardness, and stickiness; ↓ porosity and traditional bread aroma	3%	(117)
Pineapple peel	5, 10, and 15	↑ R; ↓ E	↓ specific volume	↑ hardness and gumminess; ↓ cohesiveness and springiness	↑ crust a* and b*, crumb a* and b*; ↓ crust L* and white index, crumb L* and white index	↓ colour, odour, texture, and overall assessment	5-10%	(53)
Cupuassu peel	3, 6, and 9				↑ crumb a* and b*; ↓ crust b* and crumb L*	↓ colour, aroma, texture, and flavour	6%	(80)
Pineapple core	5, 10, and 15	↑ R; ↓ E	↓ specific volume	↑ hardness and gumminess; ↓ cohesiveness and springiness	↑ crust a* and b*, crumb a* and b*; ↓ crust L* and white index, crumb L* and white index	↓ colour, odour, texture, and overall assessment	5%	(119)
Orange BP	1, 2, 3, and 5	↑ WA and AT; ↓ DS, DDT and TBD	↓ volume and specific volume	↑ firmness	↑ a* and b*; ↓ L*	↓ colour and appearance, texture, flavour, and overall acceptability	2%	(61)
Grape pomace	2, 5 and 10		↓ specific volume	↑ hardness; ↓ springiness and cohesiveness	↑ crumb a*; ↓ crust L*, a*,and b*, crumb L* and b*	↓ taste and overall acceptability	5%	(132)
Grape pomace	5, 10, and 15		↓ volume	↑ firmness	↓ L*, Chroma, and hue angle	↓ mouth feel	10%	(121)
Pumpkin pomace	5, 10, and 20			↑ hardness	↑ a* and ΔE*; ↓ L* and b*	↓ porosity, taste and aroma, and total scores	5-10%	(133)
Tomato pomace	1, 3, 5, and 7	↑ WA and SF; ↓ AT, DDT, and DS time		↓ hardness	↑ a* and b*; ↓ L*	↑ taste and flavour	5%	(59)
Pomegranate bagasse	5 and 15		↓ specific volume			↑ flavour; ↓ appearance, texture, colour, taste, mouth feel, and overall acceptability	15%	(104)
Grape seed	2.5, 5.0, 7.5, and 10.0		↑ volume; ↑ number of cells; ↓ mean cell diameter, and max cell diameter		no significant impact	no significant effects on bread quality	5%	(90)

Table 1.6. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of bread (continued)

By-product source	% added	Dough properties	Bread properties			Sensory scores (highest concentration)	Higher addition[1]	Ref.
		Rheology	Physical characteristics	Texture	Colour			
Pumpkin seed cake	5 and 10	↑ WA, DDT, and SF; ↓ DS and EL				↑ sensory bread quality	5%	(56)
Sunflower seed cake	5 and 10	↑ WA and SF; ↓ DDT, DS and EL				↑ sensory bread quality	10%	(56)
Tomato seed meal	10, 20, and 30	↑ WA, DDT, DS, and departure time; ↓ SF; ↑ gelatinization temperature; ↓ pasting peak, PV, SB, and falling number	↑ weight; ↓ volume and specific and volume			↓ texture and eating quality	10%	(109)
Walnut seed cake	5 and 10	↑ WA, DDT, and DS; ↓ EL and SF				↑ sensory bread quality	10%	(56)
Yellow linseed cake	5 and 10	↑ WA, DDT, and SF; ↓ DS and EL				↑ sensory bread quality	10%	(56)
Broad bean pod	5, 10, 15, 20, 25, and 30		↓ volume	no significant impact	↓ L*, a*, b*, and ΔE*	↓ crust and crumb colour, flavour, taste, tenderness, and overall acceptability	15%	(36)
Pea pod	5, 10, 15, 20, 25, and 30		↓ volume	↓ hardness, cohesiveness, adhesiveness, and chewiness	↓ L*, a*, b*, and ΔE*	↓ crust and crumb colour, flavour, taste, tenderness, and overall acceptability	15%	(36)
Sweet potato leaf	5, 10, and 15	↑ WA; ↓ DDT, DS, and TBD	↓ specific volume	↑ hardness and chewiness	↓ L*, a*, and b*	↓ taste, texture, colour, flavour, and overall acceptability	5%	(57)
Sweet potato stem	5, 10, and 15	↑ WA and SF; ↓ DDT, DS, and TBD	↓ specific volume	↑ hardness and chewiness	↓ L*, a*, and b*	no significant effects on bread quality	5%	(57)
Corn bran	10, 15, and 20	↑ WA, DDT, and SF; ↓ DS and TBD			↑ crumb a*, b*, and ΔE*; ↓ crumb L*,	↓ crust colour, appearance, shape and symmetry, crumb colour, structure, mouth feel, and taste-flavour	15%	(68)

Table 1.6. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of bread (continued)

By-product source	% added	Dough properties	Bread properties			Sensory scores (highest concentration)	Higher addition[1]	Ref.
		Rheology	Physical characteristics	Texture	Colour			
Oat bran	10, 15, and 20	↑ WA, DDT, and SF; ↓ DS and TBD			↑ crumb a* and ΔE*; ↓ crust ΔE*, crumb L*	↓ crust colour, appearance, shape and symmetry, crumb colour, structure, mouth feel, and taste-flavour	20%	(68)
Rice bran	10, 15, and 20	↑ DS and TBD; ↓ WA and DDT, and SF			↑ crust ΔE*, crumb a* and ΔE*; ↓ crumb L*	↓ crust colour, appearance, shape and symmetry, crumb colour, structure, mouth feel, and taste-flavour	10%	(68)
Rice bran	7.5, 15, 22.5, and 30		↓ specific volume			↓ appearance, texture, and flavour	7.5%	(106)
Rice bran	2, 5, 10, 15, and 20	↑ WA, DDT, and SF; ↓ DS; ↑ tenacity; ↓ elongation, E, and baking strength				↓ colour, appearance, texture, taste, mouth fell, and total score	10%	(108)
Rice bran	7.5, 15.0, 22.5 and 30.0		↓ specific volume		↑ a* and b*; ↓ L*	↓ crust colour, crust characteristics, brake, symmetry, crumb colour, crumb cell structure, crumb texture, aroma, taste, and total score	7.5%	(71)
Rice bran	2.5, 5.0, and 10		↓ specific volume and baking loss	↑ hardness, springiness, gumminess, and chewiness; ↓ resilience and cohesiveness	↑ a*, b*, Chroma, redness, and ΔE*; ↓ L* and whiteness	↓ taste and preference rank	10%	(126)
Wheat bran	10, 15, and 20	↑ WA , DDT, and SF; ↓ DS and TBD			↑ crust ΔE*, crumb a* and ΔE*; ↓ crust b*, and crumb L*	↓ crust colour, appearance, shape and symmetry, crumb colour, structure, mouth feel, and taste-flavour	20%	(68)
Rice bran (defatted)	5, 10, and 15	↑ WA and SF; ↓ DDT and TBD	↑ texture reading; ↓ volume			↓ crust colour and appearance, crumb colour, grain, texture, mouth fell, and overall quality	10%	(82)
Rice bran (defatted)	1, 2, 3, 4, and 6		↓ volume	↑ firmness		↓ colour, taste, odour, chewiness, and overall acceptability	3-4%	(84)
Flaxseed hull	1, 2, 3, 4, and 5		↓ volume	↑ crumb hardness		↓ aroma, texture, and taste	5%	(118)

Table 1.6. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of bread (continued)

By-product source	% added	Dough properties	Bread properties			Sensory scores (highest concentration)	Higher addition[1]	Ref.
		Rheology	Physical characteristics	Texture	Colour			
Soy hull	1.5, 3.0, 4.5, 6.0 and 7.5	↑ WA, AT, DDT, departure time, and DS; ↓ SF				↓ colour, flavour, taste, texture, chewing ability, and folding ability	4.5%	(103)
Brewer's spent grain	5, 10, 15 and 20					↓ colour, aroma, taste, texture, and acceptability	10%	(105)
Brewer's spent grain	5, 10, 15, and 20	↓ dough height; ↑ G*	no significant impact	no significant impact	no significant impact	no significant effects on bread quality	10%	(91)
Brewer's spent yeast	10		no significant impact	↑ crumb springiness and crust springiness	↑ crumb L*	↑ crumb colour ,bread odour, and crispy crust	10%	(127)
Rice straw	5		↓ volume and specific volume	↑ firmness; ↓ springiness	↑ a*, b*; ↓ L*	↓ grain, crumb colour, aroma, taste, texture, and overall acceptability	5%	(98)
Barley rootlets	5, 10, 15, and 20		↓ specific volume	↑ hardness and chewiness	↓ crust L*	↑ acidulous aroma and acidulous flavour; ↓ sweetness	10%	(85)

[1] without overall significant differences

DDT; dough development time; DS, dough stability; E, dough extensibility; EL, elasticity; G', storage modulus; G'', loss modulus; η*, complex viscosity; R, dough resistance to extension; SF, degree of softening; tan δ, damping factor; TBD, time to breakdown; WA, water absorption.

1.4.2. Sweet bakery products (Cakes, including cupcakes and muffins)

Cakes are bakery products high in fat and sugar. Base ingredients include wheat flour, shortening, sugar, leavening agents, liquid (water, milk, or buttermilk), and eggs. Cake making requires the formation of a structure that supports these ingredients, keeping it light and delicate. Cakes can be divided in two main categories: foam and shortened. In foam type cakes (angel food, sponge, chiffon), the structure and volume depend on foaming and aeration properties of eggs. In shortened type cakes (pound cake, chocolate cake, etc.) the structure results from the fat-liquid emulsion created throughout batter processing (101). Cake main attributes are structure, texture, moistness, colour (brown crust), high volume, and sweet flavour.

1.4.2.1. Wheat cake enrichment with new ingredients from food industry BP

Nutritional composition of wheat cakes enriched with potential functional ingredients from different BP is summarized on Table 1.7. As mentioned above, only data presented in fresh weight is used for comparisons. Compared to the respective control, incorporation of banana peel, mango peel, and orange peel, in wheat cakes decreases the protein content up to 75%, compared to the controls (52, 136, 137). The opposite behaviour is observed for passion fruit peel and pumpkin seed that increased protein levels from 2 to 27%, compared to respective controls (136, 138). Moreover, higher protein levels are found on cakes enriched with pumpkin seeds, while those containing mango peel and mango pulp present lower amounts (52, 136-138). Regarding TDF content in cake formulations, values rise from 1.9 to 9.2 times with BP addition, compared to the respective control (52, 136-138). Furthermore, higher TDF values are found for mango peel and pulp, and lower for pumpkin seeds and banana peel (52, 136-138). As regards fibre related nutrition claims, none of control cakes used meets the requirements for either “source of fibre” or “rich in fibre” (52, 107, 136-138). Nonetheless, wheat cake formulations with 5% mango peel, 5, 10, and 20% mango pulp, and 20% passion fruit are “source of fibre”, while wheat cakes with 30% mango pulp, 10, 20, and 30% mango peel are “high in fibre” (107). Incorporation of mango peel, passion fruit peel, and pumpkin seed in wheat cakes increases the fat content from 0.2 to 41%, compared to the controls (52, 136, 138). In wheat cakes with banana peel, mango pulp, and orange peel, changes in fat concentrations vary according to the addition levels (52, 136, 137). Banana peel, orange peel, passion fruit peel, and pumpkin seeds present higher fat values. However, fat content is also related with cake formulations that include shortening rather than the BP contribution (52, 136-138). In addition, with a fat content higher than 7 g/100 g for every cake formulation, none of them are qualified for the nutrition claim fat-free” or “low fat” (107).

Data on dough rheology changes on enriched cakes is scarce (see Table 1.8). On cakes enriched with guava pomace, WA increases and DS decreases (139). The opposite behaviour can be observed for cakes enriched with guava seeds (139). As mentioned above, changes in dough WA can be related with protein content (negative impact) and TDF chemical structure, association between molecules, particles size, and fibre porosity (positive or negative impact) (110-113). Modifications in DS can be associated to TDF composition of BP, either due to an increase in the number of hydroxyl groups available for interaction with water during dough development or due to gluten dilution (41, 56, 61, 116).

The impact of BP functional ingredients incorporation on extensional properties of wheat cakes dough, namely resistance to deformation or tenacity (P), extensibility (L), elastic resistance and extensibility balance (P/L ratio), and deformation energy (W), also varied. P increases in cakes enriched with potato peels and decreases with grape pomace addition (35, 87). P is a predictor of dough ability to retain gas and can be influenced by the interactions between wheat proteins and polysaccharides (111, 140, 141). L, an indicator of dough processing characteristics, shows a trend opposite to dough tenacity, increasing with grape pomace addition and decreasing with potato peels addition (35, 87). The P/L ratio increases in enriched wheat cakes, indicating a hardening of the dough in the presence of the BP (potato peels and grape pomace) (35, 87). Cake enrichment with potato peels and grape pomace decreases W (35, 87). Moreover, broad bean pod and pea pod incorporation in cake formulations reported by Belghith Fendri *et al.* (75) follow the same trend of potato peels for P, L, and P/L ratio, but with increasing W.

Physical characteristics, such as volume and specific volume decrease for BP enriched cakes, except for banana peels (Table 1.8) (52, 115, 121, 136, 137, 139, 142). As mentioned previously, volume and specific volume reduction can be due to gluten network influence on dough strength and gas retention (122, 123). Other physical characteristics, namely weight and height frequently increase (35, 52, 115, 136, 138, 139), which could be attributed to the higher content of DF with strong water binding properties (140).

As shown is Table 1.8, As mentioned previously, volume and specific volume reduction can be due to gluten network influence on dough strength and gas retention (122, 123). Other physical characteristics, namely weight and height frequently increase (35, 52, 115, 136, 138, 139), which could be attributed to the higher content of DF with strong water binding properties (140).

A darker colour is consistently observed for wheat cake formulations with BP addition (Table 1.8) (52, 87, 115, 121, 138, 142). As referred before, this can be due not only to Maillard and caramelization reactions, but also to the original colour of BP (134, 135). Differences on a* and b* values could likely be related to the original BP colour.

With regard to sensory analysis, scores related aroma, flavour, taste, and texture characteristics usually decrease with increasing addition of BP to cake formulations (Table 1.8) (115, 121, 136, 138, 139, 142). Nevertheless, in some formulations there is either no influence of BP incorporation on cake quality (mango peel, orange peel, potato peel, mango pulp, and grape pomace) or even better scores for some characteristics (banana peel, rice bran, apple skin, and grape pomace) (35, 52, 87, 115, 121, 136, 137, 142).

In light of all parameters analysed, especially sensory analysis, highest incorporation of BP in wheat cakes without overall significant differences was often 10%. The maximum addition value possible was 30% (pumpkin seed), while the minimum was 1.5% (Banana peel) (52, 87, 115, 137-139).

Table 1.7. Nutritional composition of sweet bakery products with incorporation of potential functional ingredients obtained from different BP (expressed in g/100 g, fresh weight basis)

By-product source	**% added**	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals***									**Ref.**
				TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Sweet bakery																			
Cakes																			
Banana peel	1.5, 3.0, and 4.5	20.67-20.75 **(20.49)**	5.98-6.05 **(6.11)**	1.59-1.61 **(0.83)**			51.67-52.77 **(52.99)**	17.85-18.79 **(18.54)**	0.97-1.35 **(1.04)**										(137)
Mango peel	5, 10, 20, and 30	35.38-35.91 **(33.25)**	0.45-1.48 **(1.80)**	4.19-8.10 **(1.23)**			45.29-49.49 **(33.54)**	7.46-9.76 **(7.42)**	2.10-2.45 **(1.99)**										(52)
Orange peel	12.5	25.55 **(27.91)**	5.46 **(5.66)**	2.84 **(1.43)**			47.91 **(46.6)**	16.95 **(17.10)**	1.29 **(1.30)**										(136)
Passion fruit peel	20	27.5 **(27.91)**	5.76 **(5.66)**	4.95 **(1.43)**			42.84 **(46.6)**	17.13 **(17.10)**	1.82 **(1.30)**										(136)
Mango pulp	5, 10, 20, and 30	29.12-34.77 **(33.25)**	0.56-2.02 **(1.80)**	3.69-6.29 **(1.23)**			6.29-53.62 **(33.54)**	8.56-9.55 **(7.42)**	1.95-2.33 **(1.99)**										(52)
Pumpkin seed	7.5, 15.0, and 30	8.50-9.14 **(8.47)**	6.90-8.14 **(6.40)**	0.76-2.40 **(0.26)**			61.47-74.05 **(77.20)**	8.65-17.36 **(6.61)**	1.14-1.49 **(1.06)**										(138)
Cupcakes																			
Guava pomace‡	5, 10, 15, and 20		6.0-8.4 **(8.97)**	1.34-4.75 **(0.70)**				7.64-8.82 **(9.79)**											(139)
Guava seed‡	5, 10, 15, and 20		7.49-9.05 **(8.97)**	1.04-4.19 **(0.70)**				10.74-13.48 **(9.79)**											(139)
Muffins																			
Grape pomace‡	5.0, 7.5, and 10.0		9.08-9.94 **(9.24)**	6.09-8.29 **(4.47)**	1.37-4.71 **(1.49)**	3.33-7.14 **(2.98)**	63.33-66.45 **(67.73)**	17.70-18.16 **(17.28)**	1.43-1.58 **(1.28)**										(87)
Brittle bakery																			
Cookies																			
Fluted pumpkin seed (defatted)	5, 10, 15, 20 and 25	8.4-8.8 **(8.2)**	16.2-23.8 **(12.3)**	1.7-1.9 **(2.0)**			50.0-58.6 **(63.4)**	13.3-13.9 **(13.2)**	1.6-1.8 **(0.9)**		670-859 **(512)**	3988-5204 **(3452)**	1701-2588 **(1091)**	1798-1986 **(1194)**			21-25 **(20)**		(64)
Millet seed coat	10 and 20	3.5-4.2 **(3.8)**	8.9-9.5 **(8.5)**	5.7-8.3 **(2.0)**	3.2-6.9 **(0.9)**	1.4-2.5 **(1.2)**	63.6-66.8 **(69.0)**	12.5-13.0 **(11.6)**	1.2-1.5 **(1.1)**		1073.0-1428.2 **(461.8)**					4.4-5.6 **(0.3)**	17.1-18.1 **(1.5)**		(73)

Values in bold within brackets are relative to control bakery product used in each study.

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

‡ Values by dry weight basis, but no information on moisture.

[a] Total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

Table 1.8. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of sweet bakery products

By-product source	**% added**	**Dough properties**	**Product properties**			**Sensory scores (highest concentration)**	**Higher addition[1]**	**Ref.**
		Rheology	Physical characteristics	Texture	Colour			
Cakes								
Banana peel	1.5, 3.0, and 4.5		↑ viscosity and volume			↑ odour, tenderness, and moistness	1.5%	(137)
Mango peel	5, 10, 20, and 30		↑ weight and density; ↓ volume and specific volume	↑ firmness, gumminess, and chewiness	↑ crumb a*and Chroma; ↓ crust L*, a*, b*, Chroma, and hue angle, crumb L*, b*, and hue angle	no significant effects on cake quality	10%	(52)
Orange peel	12.5		↓ weight			no significant effects on cake quality	12.5%	(136)
Passion fruit peel	20		↑ height ↓ weight			↓ odour, flavour, and texture	20%	(136)
Potato peel	2, 5, and 10	↑ P, P/L; ↓ L, W	↑ width and height	↑ hardness, cohesion, springiness, adhesion, chewiness, and breaking force		no significant effects on cake quality	5%	(35)
Mango pulp	5, 10, 20, and 30		↑ weight and density; ↓ volume and specific volume	↑ firmness, gumminess, chewiness, and springiness	↑ crumb a*and Chroma; ↓ crust L*, a*, b*, Chroma, and hue angle, crumb L*, b*, and hue angle	no significant effects on cake quality	10%	(52)
Pumpkin seed	7.5, 15.0, and 30.0		↑ thickness; ↓ weight loss	↑ hardness; ↓ adhesiveness and cohesiveness	↑ b*; ↓ L* and a*	↓ appearance, taste, texture, overall acceptability, and colour	30%	(138)
Rice bran	5, 10, 15, and 20		↓ specific volume and baking loss	↑ hardness and firmness; ↓ cohesiveness and springiness	↑ crust a* and crumb a*; ↓ crust L and b*, crumb L*, and b*	↑ texture and flavour; ↓ crumb colour, crust colour, taste, and overall quality	10%	(115)

Table 1.8. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of sweet bakery product (continued)

By-product source	% added	Dough properties	Product properties			Sensory scores (highest concentration)	Higher addition[1]	Ref.
		Rheology	Physical characteristics	Texture	Colour			
Cupcakes								
Guava pomace	5, 10, 15, and 20	↑ WA; ↓ DS, gluten, amylase, and retrogradation	↑ weight; ↓ volume and specific volume			↓ flavour, crumb colour, softness, moistness, tenderness, thickness, size, uniformity, crust colour, taste, and overall acceptability	5-10%	(139)
Guava seeds	5, 10, 15, and 20	↑ DS; ↓ WA, gluten, amylase, retrogradation, viscosity, and mixing time	↑ weight; ↓ volume and specific volume			↓ flavour, crumb colour, softness, moistness, tenderness, thickness, size, uniformity, crust colour, taste, and overall acceptability	5-10%	(139)
Muffins								
Apple skin	4, 8, 16, 24, and 32		↓ height and volume index	↑ firmness	↓ L*	↑ overall flavour, sweetness, and overall acceptability; ↓ moistness and cohesiveness	24%	(142)
Grape pomace	5, 7.5, and 10	↑ L and P/L; ↓ W and P		↑ hardness and chewiness; ↓ cohesiveness and resilience	↑ crumb a*; ↓ crust a* and b*, crumb L* and b*	no significant effects on muffin quality	10%	(87)
Grape pomace	5, 10, and 15		↓ volume index	↑ firmness; ↓ springiness	↓ L*, Chroma, and hue angle	↑ colour; ↓ aroma liking	10-15%	(121)

DS, dough stability; L, dough extensibility; P, dough resistance to deformation or tenacity; P/L ratio, elastic resistance and extensibility balance of flour dough; W, deformation energy; WA, water absorption

1.4.3. *Brittle bakery products (biscuits)*

Biscuits have usually high fat and sugar contents. Primary ingredients include wheat flour, shortening, sugar, and leavening agents. Biscuits dough can be classified as either soft (cookies) or hard (crackers), depending on the amount of water required for dough making. Soft dough is made with less water and relatively high levels of fat and sugar; the dough has low extensibility. Hard dough is produced with more water and has relatively low fat and sugar contents; the dough is tough and extensible (1, 143).

1.4.3.1. Wheat biscuits enrichment with new ingredients from food industry BP

Nutritional composition of biscuits enriched with potential functional ingredients from different BP is summarized on Table 1.9. As mentioned previously, only data presented in fresh weight is used for comparisons. Compared with the respective controls, protein content increases from 5 to 93% in wheat biscuit formulations with fluted pumpkin seed (defatted), millet seed coat, pigeon pea brokens, powder, and husk, pomegranate peel, and watermelon seed (37, 55, 73, 77, 144). A protein reduction of 2 to 9% is reported by Ismail *et al.* (51) for biscuits with pomegranate addition. Overall, higher protein content is found for fluted pumpkin seed (defatted), pomegranate peel and watermelon seeds, whereas pigeon pea brokens, powder, and husk, and pomegranate peel present lower contents (37, 51, 55, 73, 77, 144). BP incorporation increases (from 8 to 600%) TDF content in biscuits, except for fluted pumpkin seed (defatted) that registers a reduction from 5 to 15% (37, 51, 55, 73, 77, 144). Also, biscuits enriched with millet seed coat and pomegranate peel have higher TDF values, while lower amounts are found in biscuits enriched with fluted pumpkin seeds (defatted), pigeon pea brokens, powder, and husk, pomegranate peel, and watermelon seed (37, 51, 55, 73, 77, 144). Regarding fibre related nutrition claims, only control biscuits used by Srivastava *et al.* (144) to study pomegranate peel incorporation are already eligible for the nutrition claim “source of fibre” (107). None of the other control biscuits used meet the requirements for either “source of fibre” or “rich in fibre” (37, 51, 55, 73, 77, 107). Nonetheless, wheat biscuit formulations with 10 and 20 % millet seed coat are a “source of fibre” and “high in fibre”, respectively (73). Compared to the controls, biscuits with fluted pumpkin seed (defatted), millet seed coat, and pigeon pea brokens, powder, and husk raised the fat concentration from 1 to 12%, whereas pomegranate peel reduced it from 1 to 18% (51, 55, 73, 77, 144). The highest fat values are found for biscuits with pigeon pea brokens, powder, and husk by Tiwari *et al.* (77), and for biscuits with pomegranate peel by Ismail *et al.* (51). Generally, biscuits are characterized for their high fat content, with shortening being one of the primary ingredients. So, as would be expected, none of the biscuit formulations are qualified for the nutrition claim fat-free” or “low fat”, as their fat content is higher than 11 g/100 g (107). Biscuit enrichment with fluted pumpkin seed (defatted), millet seed coat, with pigeon pea brokens, powder, and husk, pomegranate peel, and watermelon seed increases mineral content, compared with the controls (37, 51, 55, 73, 77, 144). However, an exception is observed for Na content in biscuits with pomegranate peel, as described by Ismail *et al.* (51). Control biscuit used for the study of fluted pumpkin seed (defatted) incorporation by Giami *et al.* (55) is already a "source of P". Also, formulations with 5, 10, 15, 20, and 25% addition of fluted pumpkin seed (defatted) are a "source of Fe" (55). Additionally, biscuit 7.5% addition of pomegranate peel by Srivastava *et al.* (144) is a "source of Ca".

Dough WA increases with BP addition to wheat biscuits, except for sugarcane bagasse (9, 37, 49, 62, 74, 108, 144). As indicated previously, modifications in dough WA can be associated with protein content and or TDF chemical structure, association between molecules, particles size, and fibre porosity (110-113).

The influence of BP functional ingredients on wheat biscuit DDT, DS, and SF differs according with BP and concentrations added (9, 37, 49, 62, 73, 74, 108, 144). As mentioned above, changes in DDT and DS can be related to TDF composition of BP, as a result of the presence of hydrophilic components in BP, an increase in the number of hydroxyl groups available for interaction with water during dough development or due to gluten dilution (41, 56, 61, 114-116). Also, changes on SF depend on the tolerance towards mixing. Furthermore, no significant impact on rheological properties is observed with groundnut cake and soybean cake addition (78).

Physical characteristics of biscuits (Table 1.10) such as thickness, diameter, and spread ratio are affected by the addition of BP functional ingredients, except with pomaces from blackcurrant, elderberry, rosehip, and rowandberry, and cakes from groundnut and soybean (23, 78). Thickness and diameter are inversely related, while spread ratio depends on the proportion between diameter and thickness. Changes in these parameters can be related to gluten and TDF contents and structure (49, 144-146). As stated by Miller *et al.* (147), the formation of a continuous gluten network increases viscosity and stops the flow of cookies dough, which results in biscuits with higher thickness, smaller diameter and spread ratio. The dilution of gluten network with the addition of BP functional ingredients decreases of dough viscosity, which in turn increases diameter and spread ratio. Moreover, the preferential absorption of water by TDF results in increased numbers of hydrophilic sites available for competing for the limited free water in biscuit dough (146). This leads to a rapid partitioning of free water to these hydrophilic sites during dough mixing, resulting in increased dough viscosity. Consequently, biscuit spreading during baking is limited, resulting in cookies with an increased thickness and thus reduced diameter and spread ratio (146).

Biscuit texture parameters such as breaking strength/breaking force/fracture force and hardness are shown Table 1.10. There is a noticeable increase in these parameters along with most BP functional ingredients incorporated (9, 23, 37, 49, 60, 62, 74, 77, 79, 144). Nevertheless, there is a decrease in breaking strength and hardness with plantain peel, grape seed, fluted pumpkin seed and fluted seed (defatted) (55, 148-150). Also, for biscuits with elderberry pomace, groundnut cake, and soybean cakes, no significant impact was observed on texture parameters (23, 78). The breaking strength and hardness of biscuits are affected by the interaction between proteins, and between proteins and starch by hydrogen bonding (37, 151). If total protein content increases but gluten is reduced, or if TDF cause a dilution of gluten, there can be an increase in breaking strength and hardness (79).

A decrease in L* is usually observed for wheat biscuit formulations with BP addition (Table 1.10). As mentioned previously, Maillard and caramelization reactions, as well as the original colour of BP can explain a darker biscuit colour (134, 135). Differences on biscuits a* and b* values could likely be related to the original BP colour.

In sensory analysis, addition of BP functional ingredients to biscuit formulations usually decreases scores related with aroma, flavour, taste, and texture (Table 1.10) (8, 9, 23, 49, 51, 60, 62, 63, 65, 66, 72-74, 77-79, 81, 92, 108, 144, 148-150, 152-154). However, in some biscuit formulations there is no influence of BP addition on biscuit quality (pitaya peel, blackcurrant pomace, elderberry pomace, rosehip pomace, watermelon seed, and tomato pomace) (23, 37, 51, 155). Additionally, BP incorporation increases the scores for a number of sensory attributes in biscuits (orange pulp, guava bagasse, grape seed, and grape leaves) (orange pulp, guava bagasse, grape seed, and grape leaves) (9, 63, 148, 153).

Gathering the information obtained from all parameters analysed, especially sensory analysis, highest incorporation of BP in wheat biscuits without overall significant differences is frequently 10%. The maximum addition value possible is 25% (fluted pumpkin seed and baru cake (partially defatted)), while the minimum is 1% (grape leaves) (49, 72, 74, 77, 79, 108, 149, 150, 152, 153).

Table 1.9. Nutritional composition of brittle bakery products with incorporation of potential functional ingredients obtained from different BP (expressed in g/100 g, fresh weight basis)

By-product source	% added	Moisture	Protein	Fibre			Carbo-hydrates	Fat	Ash	Minerals*									Ref.
				TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Brittle bakery																			
Cookies																			
Pigeon pea brokens, powder and husk	5, 10, 15, 20 and 25	4.02-4.90 **(4.14)**	6.69-8.64 **(6.21)**	0.59-0.91 **(0.42)**			64.68-67.74 **(69.34)**	19.30-19.75 **(19.18)**	0.86-1.23 **(0.71)**										(77)
Pomegranate peel	1.5, 3.0, 4.5, 6.0, and 7.5	4.09-4.30 **(4.35)**	10.50-11.33 **(11.6)**	0.65-1.88 **(0.31)**			60.37-60.56 **(60.51)**	22.38-22.59 **(22.75)**	0.53-0.73 **(0.51)**		76.13-168.24 **(44.86)**	713.42-732.51 **(737.38)**	365.81-579.13 **(313.02)**		0.02 **(0.02)**	2.56-2.66 **(2.53**	0.46-0.54 **(0.44)**	0.01 **(0.01)**	(51)
Pomegranate peel	7.5	3.66 **(3.28)**	6.23 **(5.46)**	5.52 **(3.65)**				13.48 **(16.49)**	1.48 **(1.31)**		1602 **(235)**						23.1 **(14.5)**		(144)
Watermelon seed	2.5, 5.0, 7.5, and 10.0		11.44-17.48 **(10.07)**	1.3-1.4 **(1.30)**			64.05-73.16 **(74.72)**	13.05-16.23 **(13.21)**	1.68-1.88 **(1.87)**										(37)
Orange pulp‡	5, 15, and 25		5.23-5.63 **(5.54)**	5.47-14.7 **(2.10)**	2.34-7.21 **(1.17)**	3.13-7.50 **(0.93)**	60.97-69.82 **(76.63)**	17.56-18.28 **(17.49)**	0.93-1.36 **(1.24)**										(9)
Grape pomace‡	10, 20, 30			6.49-11.0 **(3.44)**	5.54-9.13 **(2.71)**	0.95-1.90 **(0.73)**													(88)
Soybean cake‡	20		15.3 **(8.1)**	12.7 **(3.5)**	7.8 **(2.6)**	4.9 **(0.9)**		13.1 **(12.7)**	1.22 **(1.01)**										(78)
Groundnut cake‡	20		13.7 **(8.1)**	12.4 **(3.5)**	7.4 **(2.6)**	5.0 **(0.9)**		14.5 **(12.7)**	1.21 **(1.01)**										(78)
Mango peel†	2.5, 5.0, 7.5, and 10.0			11.0-20.7 **(6.5)**	7.40-12.50 **(3.67)**	3.60-8.20 **(2.80)**													(49)
Pitaya peel†	5, 10, and 15	1.98-4.66 **(4.35)**	6.85-7.04 **(6.73)**	0.73-2.00 **(0.07)**			59.42-61.32 **(60.43)**	25.87-26.80 **(26.62)**	2.39-3.11 **(2.17)**										(155)
Plantain peel†	5, 10, and 15	6.25-6.55 **(5.57)**	8.99-10.25 **(11.32)**	15.78-36.74 **(13.26)**	10.54-26.87 **(8.43)**	5.25-9.87 **(4.83)**	48.16-56.25[a] **(58.41)**	22.94-23.16 **(23.22)**	1.23-1.90 **(1.08)**										(149)

Table 1.9. Nutritional composition of brittle bakery products with incorporation of potential functional ingredients obtained from different BP (expressed in g/100 g, fresh weight basis) (continued)

By-product source	**% added**	**Moisture**	**Protein**	**Fibre**			**Carbo-hydrates**	**Fat**	**Ash**	**Minerals***									**Ref.**
				TDF	IDF	SDF				Mg	Ca	Na	K	P	Mn	Zn	Fe	Cu	
Brittle bakery																			
Carrot pomace†	4, 8, and 12	3.82-4.49 **(2.57)**	6.80-7.15 **(7.25)**	4.98-10.18 **(2.48)**	2.64-5.64 **(1.39)**	2.34-4.54 **(1.09)**		23.83-24.94 **(24.87)**	1.18-1.45 **(0.77)**										(65)
Tomato pomace†	5, 10, 15, 20, and 25	2.24-3.39 **(2.49)**	5.50-6.87 **(5.19)**					21.80-21.85 **(21.71)**	4.844-4.927 **(4.883)**										(66)
Mango kernel (defatted) †	5, 10, 15, 20, 25 and 30	3.41-3.59 **(3.46)**	6.22-6.50 **(6.62)**	0.60-1.05 **(0.53)**				24.10-24.46 **(24.46)**	0.89-1.14 **(0.84)**										(8)
Flaxseed meal (defatted) †	10, 20, and 30	2.5-2.7 **(2.4)**	7.4-12.8 **(5.1)**	4.5-7.7 **(0.7)**				14.7-19.1 **(18.4)**	1.1-2.3 **(0.6)**										(152)
Soybean meal (defatted) †	10, 20, 30, and 40	2.5-2.9 **(2.4)**	9.3-15.9 **(5.1)**	3.3-7.5 **(0.7)**				13.8-18.6 **(18.4)**	1.2-2.4 **(0.6)**										(152)
Sunflower meal (defatted) †	10, 20, 30, and 40	2.6-3.1 **(2.4)**	8.8-13.1 **(5.1)**	3.6-8.2 **(0.7)**				14.7-17.2 **(18.4)**	1.1-2.4 **(0.6)**										(152)
Rice bran (defatted) †	5, 10, and 15	5.48-6.52 **(5.13)**	11.13-13.63 **(10.20)**					15.11-18.02 **(14.20)**		59.93-99.04 **(46.78)**	39.18-71.51 **(25.84)**				5.18-8.08 **(4.36)**	9.35-12.28 **(7.89)**	32.92-41.55 **(29.75)**		(81)
Rice bran (defatted) †	10, 20, 30, 40, and 50			4.25-11.01 **(2.56)**						682.9-2988.7 **(106.4)**	820.8-1906.0 **(549.5)**	1004.4-1773.0 **(1965.1)**	1170.4-4070.1 **(445.5)**						(154)
Brewer's spent grain†	3, 6, 9, 12, and 15		1.68-1.86 **(1.64)**					2.12-2.31 **(2.15)**	1.66-4.59 **(4.52)**						0.4-0.9 **(0.4)**	0.3-0.6 **(0.5)**	1.2-1.8 **(0.9)**	0.3-0.4 **(0.1)**	(92)
Crackers																			
Tomato pomace†	4, 8, and 12		7.63-7.82 **(7.35)**	5.3-854 **(1.86)**	3.48-6.04 **(0.84)**	1.82-2.50 **(1.02)**		16.75-18.06 **(16.50)**	1.52-1.69 **(1.27)**	283.9-400.7 **(185.0)**	356.2-569.9 **(213.9)**		2108.9-3090.0 **(1491.1)**	2123.1-2314.2 **(1871.8)**	12.3-23.0 **(9.7)**	11.0-22.6 **(8.8)**	12.6-22.1 **(8.9)**		(58)

Values in bold within brackets are relative to control bakery product used in each study.

[*] mg/kg.

[†] No indication if data is expressed in fresh or dry weight basis.

[‡] values by dry weight basis, but no information on moisture.

[a] total carbohydrate

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

Table 1.10. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of brittle bakery products

By-product source	% added	Dough properties	Product properties			Sensory (highest concentration)	Higher addition[1]	Ref
		Rheology	Physical characteristics	Texture	Colour			
Cookies								
Mango peel	2.5, 5.0, 7.5, and 10.0	↑ WA, DDT, and SF; ↓ DS	↓ diameter and thickness	↑ breaking strength	↓ L*, a*, and b*	↓ crust colour, crust appearance, crumb colour, texture, taste/flavour, and overall quality	10%	(49)
Pitaya peel	5, 10, and 15		↑ diameter; ↓ thickness		↑ a*; ↓ L* and b*	no significant effects on cookies quality	15%	(155)
Plantain peel	5, 10, and 15		↑ thickness; ↓ spread ratio	↓ breaking strength	↓ L*, a*, b*, and browning index	↓ colour and taste	10%	(149)
Pomegranate peel	1.5, 3.0, 4.5, 6.0, and 7.5					↓ taste, colour, crispiness, texture, and overall acceptability	6%	(51)
Pomegranate peel	2.5, 5.0, 7.5, and 10.0	↑ WA; ↓ DS; ↑ PT, PV, HPV, and SB; ↓ CPV, and BD	↓ spread ratio	↑breaking strength		↓ surface colour, surface character, crumb colour, texture, mouth feel, flavour, and overall quality	7.5%	(144)
Orange pulp	5, 15, and 25	↑ WA, DDT, DS, and departure time; ↓ SF	↑ in weight and thickness; ↓ diameter, expansion factor, and specific volume	↑ breaking strength		↑ appearance; ↓ flavour, texture, and general acceptance	15%	(9)
Blackcurrant pomace	20		no significant impact	↑ breaking force	↑ a* and b*; ↓ L*	no significant effects on cookies quality	20%	(23)
Elderberry pomace	20		no significant impact	no significant impact	↑ b*; ↓ L*	no significant effects on cookies quality	20%	(23)
Rosehip pomace	20		no significant impact	↑ breaking force	↑ a* and b*; ↓ L*	no significant effects on cookies quality	20%	(23)
Rowanberry pomace	20		no significant impact	↑ breaking force	↑ a* and b*; ↓ L*	↓ total score for organoleptic characteristics	20%	(23)

Table 1.10. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of brittle bakery products (continued)

By-product source	% added	Dough properties	Product properties			Sensory (highest concentration)	Higher addition[1]	Ref
		Rheology	Physical characteristics	Texture	Colour			
Cookies								
Carrot pomace	4, 8, and 12					↓ taste, texture, and overall acceptability	8%	(65)
Tomato pomace	5, 10, 15, 20 and 25		↑ diameter and spread factor; ↓ thickness		↑ a* and b*; ↓ L*	↓ taste, colour, appearance, and overall acceptability	5%	(66)
Guava bagasse	5, 10, 15, and 20					↑ flavour, texture, and overall appearance	20%	(63)
Sugarcane bagasse	5, 10, and 15	↑ DDT and SF; ↓ WA and DS	↓ width, thickness, and spread ratio	↑ breaking strength;	↑ a*and b*; ↓ L* and ΔE*	↓ colour, surface character, crumb colour, crumb texture, taste, and overall quality	10%	(74)
Cashew apple bagasse	5, 10, 15, and 20					↓ flavour, texture, and overall appearance	15%	(63)
Watermelon seed	2.5, 5.0, 7.5, 10.0	↑ WA, arrival time, and SF; ↓ DDT, stability time, and E force and distance		↑ fracture force	↑ ΔE*	↓ texture and overall acceptability	7.5%	(62)
Watermelon seed		↑ WA, arrival time, SF, and DS; ↓ DDT, stability time, and E force and distance; ↑ PT; ↓ PV, HPV, CPV, BD, SB		↑ fracture force	↑ ΔE*	no significant effects on cookies quality	7.5%	(37)
Fluted pumpkin seed	5, 10, 15, 20 and 25		↑ weight; ↓ diameter, height, and spread ratio	↓ hardness		↓ colour, texture, flavour, and overall acceptability	15-25%	(150)
Grape seed	10, 20 and 30		↑ diameter and spread ratio; ↓ thickness	↓ breaking strength	↑ a*; ↓ L* and b*	↑ brown flavour and fruity-acidic flavour; ↓ colour, texture, taste, and overall acceptance	10%	(148)

Table 1.10. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of brittle bakery products (continued)

By-product source	% added	Dough properties	Product properties			Sensory (highest concentration)	Higher addition[1]	Ref
		Rheology	Physical characteristics	Texture	Colour			
Cookies								
Fluted pumpkin seed (Defatted)	5, 10, 15, 20 and 25		↑ weight; ↓ diameter, height, and spread ratio	↓ hardness		↓ colour, texture, flavour, and overall acceptability	15%	(64)
Mango kernel (defatted)	5, 10, 15, 20, 25, and 30		↑ thickness; ↓ diameter, spread factor, and hardness		↓ L*, a*, b*, Chroma, and hue	↓ colour, flavour, texture, aroma, crispiness, and overall acceptability	15-20%	(8)
Groundnut cake	10, 15, and 20	no significant impact	no significant impact	no significant impact	no significant impact	↓ colour, surface character, crumb colour, crumb texture, mouth feel, and overall quality	20%	(78)
Soybean cake	10, 15, and 20	no significant impact	no significant impact	no significant impact	no significant impact	↓ colour, surface character, crumb colour, crumb texture, mouth feel, and overall quality	20%	(78)
Baru cake (partially defatted)	25, 50, 75, and 100			↑ hardness and fracturability		↓ overall acceptance, appearance, flavour, and texture	25%	(79)
Flaxseed meal (defatted)	10, 20, 30 and 40		no significant impact		↑ a* and b*, ↓ L*	↓ top grain, appearance, texture, flavour, and overall acceptability	10%	(152)
Soybean meal (defatted)	10, 20, 30 and 40		↓ spread ratio		↑ a*, ↓ L* and b*	↓ top grain, appearance, texture, flavour, and overall acceptability	10%	(152)
Sunflower meal (defatted)	10, 20, 30 and 40		no significant impact		↓ L*, a*, and b*	↓ top grain, appearance, texture, flavour, and overall acceptability	10%	(152)
Rice bran	5, 10, 15, and 20		↑ thickness; ↓ width and spread factor			↓ colour, taste, flavour, and overall acceptability	15%	(81)

Table 1.10. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of brittle bakery products (continued)

By-product source	% added	Dough properties	Product properties			Sensory (highest concentration)	Higher addition[1]	Ref
		Rheology	Physical characteristics	Texture	Colour			
Cookies								
Rice bran	2, 5, 10, 15, and 20	↑ WA, DDT, DS, and SF;; ↑ P; ↓ elongation, L, and baking strength				↓ colour, appearance, crumb colour, texture, taste, mouth fell, and total score	10%	(108)
Rice bran	5, 10, and 15		↑ thickness, weight, and fracture strength; ↓ diameter, spread ratio			↓ colour, appearance, flavour, texture, taste, and overall acceptability	10%	(72)
Rice bran (defatted)	10, 20, 30, 40 and 50		↑ thickness; ↓ width and spread ratio			↓ colour, flavour, taste, texture, crispness, and overall acceptability	20%	(154)
Brewer's spent grain	3, 6, 9, 12, and 15		↓ spread ratio			↓ appearance, taste, colour, flavour, and overall acceptability	6%	(92)
Celery spent residue	5.0, 7.5, and 10		↑ thickness ; ↓ diameter and spread ratio	↑ breaking strength	↓ L*, a*, b*	↓ overall quality score	7.5%	(60)
Grape leaves	1		↑ width and spread ratio; ↓ weight and thickness			↑ colour, texture and taste; ↓ surface character, crumb colour, mouth feel, and total score	1%	(153)
Millet seed coat	10 and 20	↑ SF; ↓ farinograph quality number, DS, and DDT; ↑ gelatinization temperature; ↓ PV	↑ thickness; ↓ diameter		↑ ΔE*; ↓ L*, a*, b*	↓ colour, surface characteristics, crumb colour, texture, eating quality, and total score	20%	(73)

Table 1.10. Influence of potential functional ingredients obtained from different BP on dough, final product and sensory characteristics of brittle bakery products (continued)

By-product source	**% added**	**Dough properties**	**Product properties**			**Sensory (highest concentration)**	**Higher addition**[1]	**Ref**
		Rheology	Physical characteristics	Texture	Colour			
Cookies								
Pigeon pea brokens, powder and husk	5, 10, 15, 20 and 25		↑ thickness and weight; ↓ diameter and spread ratio	↑ hardness		↓ surface colour, surface characteristics, distribution of gas cell, crumb colour, texture, mouthfeel, flavour, and total score	10%	(77)
Crackers								
Tomato pomace	4, 8, 12				↑ a* and b*; ↓ L*	no significant effects on crackers quality	12%	(51)

[1] without overall significant differences

BD, breakdown (PV-HPV); CPV, cool paste viscosity (final viscosity at 50 °C); DDT; dough development time; DS, dough stability; HPV, hot paste viscosity (minimum viscosity at 95 °C); L, dough extensibility; P, dough resistance to deformation or tenacity; PT, pasting temperature; PV, peak viscosity; SB, Set back (CPV-HPV); SF, degree of softening; WA, water absorption

1.5. Summary of the effects of BP functional ingredients on bakery products nutritional profile

In recent years, there has been a growing demand on healthy nutrition, and consequently, the development of a wide variety of food products with nutritional benefits. BP functional ingredients can be used for improving nutritional quality of traditional bakery products.

Data available on fresh weight composition of different bakery products was gathered and used for average macronutrient calculation by categories (i.e. bread, cakes, and biscuits), shown in 1.1.

For breads (Figure 1.1.i), incorporation of BP functional ingredients results mainly on a decrease of carbohydrates (from 70 to 48%) and increase of moisture (from 11 to 28%), compared to the controls. As seen in Figure 1.1.ii and iii, the major effect of BP functional ingredients addition to cakes and biscuits is the increase of dietary fibre (up to 5 times and 3 times, respectively), compared to the respective controls.

Overall, enrichment with BP functional ingredients seems to benefit breads at a larger extent than cakes and biscuits. Moreover, nutritional profile of enriched cakes and biscuits cannot compete with the one presented by breads.

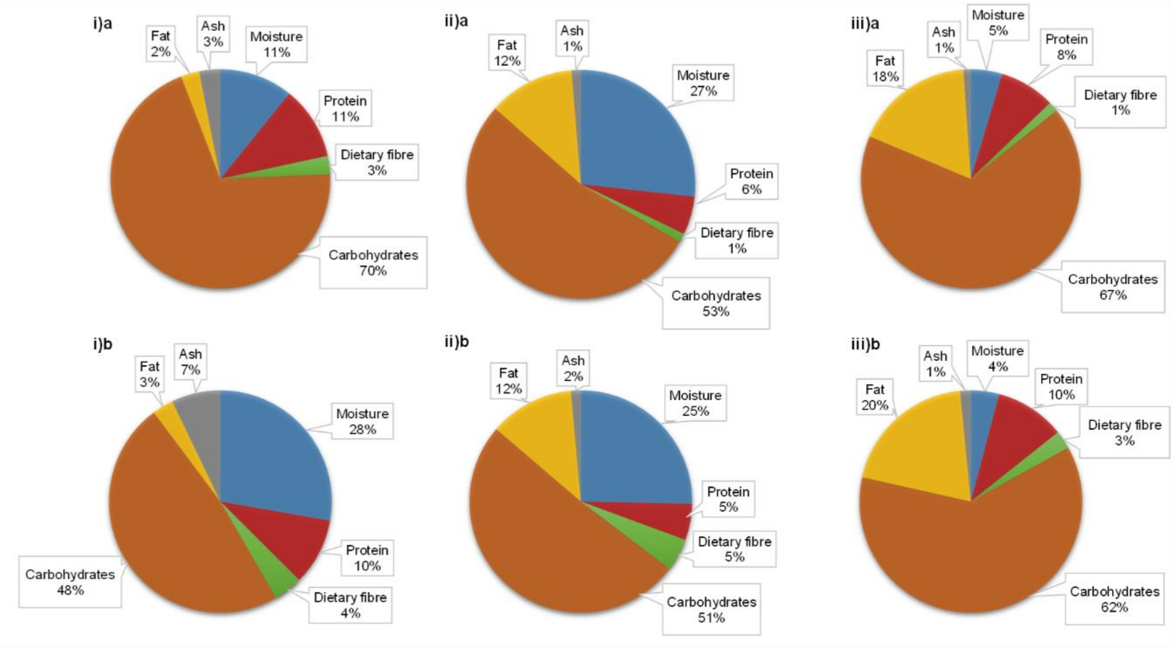

Figure 1.1. Average macronutrient composition of different bakery products categories (per 100 g, fresh weight basis). **a,** control; **b,** enriched; **i)** breads; **ii)** cakes; **iii)** biscuits

Based on data from: Ho *et al.* (155), Staichok *et al.* (156), Eshak (50), de Oliveira *et al.* (136), Bhol *et al.* (104), Aksoylu *et al.* (148), Prokopov *et al.* (157), Sudha *et al.* (158), Srivastava *et al.* (144), Ismail *et al.* (51), Bitencourt *et al.* (138), Sodchit *et al.* (137), Salgado *et al.* (80), Noor Aziah *et al.*

(52), Tiwari *et al.* (77), Giami *et al.* (55), Ameh *et al.* (102), Yadav *et al.* (72), Krishnan *et al.* (73), Soares Júnior *et al.* (106), Fărcaş *et al.* (105)

1.6. Concluding remarks

Physico-chemical composition of BP functional ingredients largely varies according to their source and can stand out due to content on TDF, protein, and/or minerals. Overall, incorporation of BP functional ingredients can improve nutritional characteristics of bakery products. However, they can also impair some functional and sensory properties. A balance on BP incorporation has to be found, in order to obtain healthier bakery products that can compete in sensory characteristics with the traditional ones.

PART II

BREWER’S YEAST BY-PRODUCTS IMPACT ON WHEAT BREAD CHARACTERISTICS

CHAPTER 2

Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of homemade bread

Abstract

The impact of bread fortification with dry spent yeast from brewing industry on physical, chemical and sensorial characteristics of homemade bread was evaluated with the goal of increasing its β-glucans content. A serving of 50 g of bread fortified with dry spent yeast increased β-glucans intake from 65 mg to 125 mg, which is within the guidelines suggested by European Food Safety Authority. Although, this fortification darkened the crumb, increased crumb and crust springiness and had impact on volatile profile, concerning key odours only hexanal presented a significant increase in fortified bread. Two machine types (with 1 or 2 paddles) were tested but had a minor impact on bread characteristics. Descriptive analyses performed by a trained panel shown no significant differences in sensorial attributes. Dry spent yeast can be used as ingredient in homemade bread to increase β-glucans intake, and contribute to valorisation of this brewing by-product.

Keywords: homemade bread, β-glucans, brewing by-products, fibre fortification, key odours, sensory analyses

2.1. Introduction

Nowadays consumers search for producing tasty foods that will contribute to improve or maintain their health, due to the addition of new ingredients that contain bioactive compounds. These new ingredients must comply with nutritional, energy and safety needs of consumers and also with legislation. An additional challenge is to find these innovative ingredients in a cost-effective and sustainable way.

Brewing spent yeast is the second major by-product from the brewing industry. It is low in calories, fat, sodium and carbohydrates, but can be a valuable source of cheap fibre, mainly β-glucans, vitamins, and chromium, which will result profitable for both the industry and the consumer (24-26).

β-Glucans are natural cell wall polysaccharides and are a major structural component of baker's and brewing yeast. They consist of β-(1→3) and β-(1→6) linked glucose polymers (24, 159, 160).

Long chain, water-insoluble and nondigestible yeast β-glucans are able to modulate mucosal immunity of the intestinal tract, facilitates bowel motility and can be used in obstipation, among other intestinal problems (161). Also, β-(1→3)-glucan have proved to reduce blood serum cholesterol, showing both hypolipidemic and hypoglycemic effects in animal and human studies (162-164).

The European Food Safety Authority (EFSA) has already approved the use of Saccharomyces β-glucans – referred to as "yeast beta-glucans" - as a new ingredient and suggests a use ranging between 50 and 200 mg of "yeast beta-glucans" per serving (165). However, studies about the impact of this functional ingredient in food quality, namely bread, are scarce (160).

Bread is one of the most consumed foods in the world and an important source of nutrients, namely proteins, starch, dietary fibres, vitamins, micronutrients and antioxidants. Since bread is consumed in a daily base there is a growing interest on the incorporation of functional ingredients in bread to reply consumer's demands on healthy nutrition (166-168). Additionally, interest in new sources of dietary fibre and bioactive compounds has grown rapidly. Fibres in various forms have been previously used in breadmaking to increase bread nutritional value (169, 170). Novel sources of dietary fibre to incorporate in bakery products, such as those generated from by-products received much attention (40, 170-174).

However, the addition of new ingredients to bread can influence its quality. The assessment of bread quality is a complex process usually defined according to sensory parameters, volume, texture, colour, and flavour (175-179). The volatiles that contribute to bread aroma are one of the most important parameters influencing consumer acceptance. More than 300 volatile compounds have been identified in bread (176, 180-182). From the wide variety of chemical compounds that contribute to bread flavour, the most relevant are the aldehydes, alcohols, ketones, esters, acids, pyrazines, and pyrrolines, as well as hydrocarbons, furans, and lactones (181, 183). Not all these compounds have the same degree of influence on the flavour, but there are key odorants that have a marked influence on both crust and crumb, namely, 3-methyl-1-butanol, 3-methyl-butanal, 2-phenylethanol, 2,3-butanedione, hexanal, and *trans* 2-nonenal (176, 182).

Concerning bread manufacture, nowadays, domestic bread making machines incorporating one or two paddles offer an alternative and facilitate the lives of those who like to bake their own bread at home. Flour mixtures added of salt, sugar, and yeast are commercially available and can include bioactive ingredients.

The objective of this work was to prepare homemade bread with enhanced nutritional benefits, due to high content of β-glucans, through fortification with dry spent yeast from brewing industry and investigate the impact on physical parameters, volatile composition and sensorial characteristics. For this purpose several sub-goals were established, namely, (i) quantification of β-glucans in dry spent yeast and also in fortified and non-fortified breads; (ii) assessment in homemade breads (prepared in one paddle and two paddles domestic machines) of the influence of spent yeast addition in bread volume, texture, colour and impact on volatile profile and quantification of relevant key odour bread compounds; (iii) effect on sensory characteristics of homemade bread.

2.2. Material and methods

2.2.1. Chemicals and standards

All solvents, reagents and standards used in this study were of analytical grade. Methanol was supplied by Merck (Darmstadt, Germany). The standards used for quantification and or confirmations of the identity of the volatiles were: 2-phenylethanol, ethanol, 3-methyl-1-butanol, 2-methyl-1-butanol, hexanal, 3-methylbutanal, *trans*-2-nonenal, 2-methyl-1-propanol, hexanol, octanol, 2-methyl-butanal, heptanal, benzaldehyde, pentane, hexane, 2-propanone, 2-butanone, octanoic acid, methyl acetate, ethyl acetate, methyl butyrate, furfural, furfuryl alcohol, acetylfuran, 2-pentylfuran. In addition 2-ethylbutiric acid was used as internal standard. Standards were supplied by Sigma-Aldrich (St. Louis MO).

Standard sock solutions of 2-phenylethanol, 3-methyl-1-butanol, hexanal, 3-methylbutanal, and *trans*-2-nonenal, were prepared by injecting 10 µl of refrigerated standard with a syringe through the septum of a 15 ml HS vial filled with 10 ml of methanol and sealed. The exact weight of methanol and standard was recorded, expressing the concentration in mg ml-1 and taking into account the density of methanol. This solution is stable for at least two weeks if kept at 4 ºC. Diluted standard solutions were prepared in methanol and kept in refrigerate and protected from light.

2.2.2. Preparation of dry spent yeast from brewing industry

The spent yeast biomass used to produce Lager beer (*Saccharomyces pastorianus*) was supplied by a local beer industry. It was washed three times with deionized water to remove beer residues, at a ratio of 1:3 (w/v) (yeast biomass: water). Between each wash it was centrifuged at 10,000 g, 4°C, 5 min. The resulting yeast cell pellet was slowly dried in an dynamic oven at 30ºC for 48 h and milled with a Knife Mill Grindomix GM200 (Retsch GmbH, Germany). β-Glucan content was quantified using the "Enzymatic yeast beta-glucan – assay procedure" (Megazyme International Ireland Ltd., Bray, Ireland).

2.2.3. Breadmaking

White bread baking mix flour supplied by Cerealis-Nacional (Maia, Portugal) was selected to perform this study. The mix contains wheat flour, dried yeast, dextrose and salt. Control bread samples were prepared as described in product label, thus 500 g of this mix added of 320 ml of water and baked using two different types of domestic bread machines: A - Moulinex with 2 paddles and 1650W power (Groupe SEB, France); B - Clatronic with 1 paddle and 600 W power (Clatronic International BBA GmbH, Germany). The conditions of leaving and baking were the same for the two machines used. Fortified breads were prepared in a similar way as control breads but 10 g of dry spent yeast were added to 500 g of the commercial mix flour. Total bread making time was 3 h and bread was cooled at room temperature during 90 min before physical and volatile analyses. Twelve control breads were prepared for the six tests carried out for optimization of HS-SPME-GC/MS conditions, for this purpose only machine A was used. With regard to analyses of volume, texture, colour and volatile profile, six breads were prepared in machine A, three control breads (coded as CBA) and three fortified breads with addition of 10 g of dry spent yeast (coded as FBA). Similarly six breads were prepared in machine B to obtain CBB and FBB samples. Concerning quantification of selected volatiles and sensorial analyses another batch of 3 CBA, 3 FBA, 3 CBB and 3FBB was prepared. Moreover, samples of control and fortified breads were prepared to perform the panel training.

2.2.4. Evaluation of bread volume, texture and colour

Bread weight and volume were measured 90 min after removal from bread making machine. The bread specific volume (SV) was measured using a seed displacement method (Cerealis internal method) and the following formula

$$SV = Sx1.35/P \tag{2.1}$$

P (g) Bread weight; S (g) Weight of the displaced seeds, 1.35 (cm^3/g) Specific volume of the *Phalaris canariensis* seeds, SV (cm^3/g) Specific volume of the bread.

For texture and colour assays, the bread was cut in half and the measurements were performed in two different zones of the bread, crumb and side crust. Texture analysis was performed using a texture analyser (model TA-XT-2iHR, Stable MicroSystems, Ltd., Surrey, U.K.) containing 5 kg of load cell. Calibrations were performed with 2 kg of load cell. Exponent Software supplied with the instrument was used. Bread crust and crumb were subjected to a 30 mm penetration depth through a two-cycle sequence using a spherical probe (25 mm in diameter) (Cyl. Perspex P/25, Stable MicroSystems, Ltd.) with a cross head speed of 1 mm/s at three different points for either crumb or crust. The texture parameters were: hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); springiness (m) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required for the first compression and A2 the total energy required for the second compression); chewiness (J) = the work needed to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness). A Minolta CR-300 colorimeter (Minolta, Ramsey, NJ) with illuminate D65, a 0° standard observer and a 2.5 cm port/viewing area was used for measurement of colour in the CIElab system – lightness (L*), redness (a*) and yellowness (b*). The colorimeter was standardized with a white tile having the following values: L* = 93.5, a* = 1.0 and b* = 0.8 before measurement in bread crust (n=3) and crumb (n =3).

2.2.5. Volatile compounds analysis by HS-SPME-GC/MS

The first step was optimization of extraction conditions for HS-SPME-GC/MS analysis of volatiles, thus, a slice (1.5 cm of thickness) of each of the twelve test samples of bread was collected after 90 min of cooling and entirely crushed, including the crust and the crumb. The influence of water/salt addition and SPME extraction time and temperature were evaluated using the method described by Petisca *et al.* (184), briefly 2 g of bread were placed in a 50-mL vial. After sealing the vials were kept at -4 ºC during 10 min, and into an ultrasonic cleaner (FUNGILAB, Portugal) during 15 min, favouring the equilibrium between the matrix and the HS (184). Extraction of volatile compounds was performed using a CAR–PDMS SPME fibre (75 µm thickness, Supelco Co., Bellefonte, PA, USA). The fibre was inserted into the sample vial through the septum and exposed to the HS at variable time and temperature. Constant magnetic stirring at 600 rpm was performed at this stage. Thereafter, the SPME fibre was inserted and desorbed for 10 min at 280 ºC, in the split-less mode, with 1 ml/min flow.

Six tests were performed in duplicate: test 1 – SPME extraction at 37 ºC during 40 min; test 2 – addition of 3 g of salt, SPME extraction also at 37 ºC during 40 min; test 3 – addition of 5 ml of water and 3 g of salt, 37 ºC during 40 min; test 4 – addition of 5 ml of water and 3 g of salt, 50 ºC during 40 min; test 5 – addition of 5 ml of water and 3 g of salt, 50 ºC during 60 min; test 6 – addition of 10 mL of 20% NaCl solution (pH 3 with 0,05M citric acid), 50 ºC during 60 min.

After selection of HS-SPME-GC/MS analytical conditions (test 6), the next step was the evaluation of impact on volatile profile of spent yeast addition followed by quantification of relevant aroma compounds on control (3 CBA and 3 CBB) and fortified breads (3 FBA and 3 FBB).

2.2.6. GC–MS conditions

Chromatographic analysis was performed using an Agilent 6890 gas chromatograph (Agilent, Avondale, PA, USA) coupled to a mass selective detector (Agilent 5973). Volatiles were separated on a 5% Fenilmetilpolisiloxano (SPB-5), bonded phase fused-silica capillary column (Hewlett–Packard, Palo Alto, CA, USA; 60 m - 320 lm i.d., film thickness 1 lm), operating at 80 kPa column head pressure, resulting in a flow of 1 ml min^{-1} at 40 ºC. The oven temperature program was isothermal for 5 min at 40 ºC, raised to 135 ºC at a rate of 3 ºC min^{-1} and then raised to 220 ºC at 20ºC min^{-1}. The transfer line to the mass spectrometer was maintained at 250 ºC. Mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 2056 V, collecting data at a rate of 1 scan s^{-1} over the m/z range 30–500. Volatile compounds were identified by comparison with the mass spectrum of standards and/or from Nist 98 data bank (NIST/EPA/NISH Mass Spectral Library, version 1.6, U.S.A.). Compounds were also detected by m/z characteristic ion.

2.2.7. Calibration curves for quantification of key odorants

Five volatile compounds, 2-phenylethanol, 3-methyl-1-butanol, hexanal, 3-methylbutanal, and *trans*-2-nonenal, which are described as bread key odorants were quantified by external calibration curve method. Standard solutions for calibration curves were prepared in methanol. 2-ethylbutiric acid was used as internal standard. 100 µl of standard solution mixture (concentration ranged between 0.025 and 2 mg/L for 2-phenylethanol, 0.1 and 10 mg/L for 3-methyl-1-butanol, 0.01 and 1 mg/L for hexanal, 0.1

and 2 mg/L for 3-methylbutanal, 0.01 and 0.05 mg/L for *trans*-2-nonenal) and 100 µl of internal standard solution (0.5mg/L) were placed in a 50 ml HS vial containing 9,8 mL of a 20% NaCl solution (pH3 with 0.05 M citric acid). After sealing the vials were kept at -4 ºC during 10 min, and into an ultrasonic cleaner during 15 min. HS-SPME extraction was performed at 50 ºC during 60 min. The quantity of each volatile compound in bread samples was calculated by using the calibration curves constructed (volatile peak area/internal standard peak area vs volatile concentration).

2.2.8. Bread sensory analysis

The sensory panel was composed of 14 master students from the University of Porto who had sensory analysis in their curriculum and expressed an interest and disposition to undertake the work. The panel was trained for descriptive analysis according to the guidelines in the ISO (185) to evaluate the influence of dry spent yeast addition and machine type on the bread sensory characteristics. In session 1, panellists proposed various attributes to start the panel training, tasted control bread samples and redundant descriptive terms were removed (186). Session 2 was designed to establish ballot anchors for selected attributes that were fitted on an unstructured scale (7 points). To assist panellists different reference bread samples were used for training each attribute and respective intensity as summarized in Table 2.1. In session 3, the ballots were tested individually by panellists using unknown representative samples. The panel agreed on 16 attributes to constitute the breads descriptive profile. Each assessor received a list of sixteen attributes: “Crust colour”, “Crumb colour”, “Odour intensity”, “Bread odour”, “Strange odour”, “Elasticity of the crumb (in the fingers)”, “Aroma intensity”, “Bread aroma”, “Strange aroma”, “Salty”, “Astringent”, “Bitter”, “Taste persistence”, “Crispy crust”, “Adhesiveness (mouth)” and “Overall assessment”. Analysis of variance (187) was performed on data collected on two more training sessions, and panellists’ deviations were assessed to determine whether additional training was needed. In evaluation sessions approximately 50 g of each sample (a slice with 1.5 cm of thickness) including the crust and crumb, was presented to assessors in a 3-digit coded glass covered with a glass lid. Assessment was carried out individually under white light at room temperature. Each assessor was provided with filtered water and asked to cleanse their palate between tastings. Control (3 CBA and 3 CBB) and fortified breads (3 FBA and 3 FBB) were analysed over three sessions.

Table 2.1. Preparation of reference bread samples used for panellist training of attributes and respective scale

Bread attributes	**Preparation of reference samples**	**Range**	**Scale**
Crust colour Crumb colour	Control bread added with oat flour	0 g of oat 50g of oat 100 g of oat	3 4 5
Odour intensity Bread odour Aroma intensity Bread aroma Elasticity of the crumb	Control bread with different cooling periods	10 min 90 min 180 min	6 4 3
Adhesiveness (mouth)	Control bread with different cooling periods	10 min 90 min 180 min	4 2 1
Crispy crust	Control bread with different selection of machine baking time	Low Medium High	1 3 5
Strange odour Strange aroma Astringent Bitter Taste persistence	Control bread with replacement of part of water by liquid milk whey	0 ml of whey 50 ml of whey 100 ml of whey	1 3 5
Salty	Control bread with increased content of salt	mix flour 2 g of salt 5 g of salt	1 3 6

2.2.9. Statistical analysis

The mean averages and standard deviations were calculated for each experimental parameter. The effect of fortification with β-glucans by addition of dry spent yeast and the effect of machine type were analysed by two-way ANOVA. Statistical analyses were performed with SPSS for Windows, version 20 (SPSS, Chicago, IL).

2.3. Results and discussion

2.3.1. β-glucans quantification in dry spent yeast, in fortified and non-fortified breads

The β-glucan contents expressed in (w/w dry weight) was 8% in dry spent yeast, 0.13% in non-fortified bread and 0.25% in bread fortified with 10 g of dry spent yeast. Concerning the β-glucan intake from a serving of bread (50 g) 65 mg were quantified in non-fortified bread and the intake increases to 125 mg in bread fortified with 10 g of dry spent yeast.

These contents were within the range suggested by EFSA guidelines of 50 to 200 mg of Saccharomyces β-glucans per serving (165).

2.3.2. Influence of dry spent yeast addition in bread volume, texture, and colour

Mean weight of CBA samples (709 ± 12 g) was similar to mean weight from CBB samples (709 ± 2.9 g). As expected fortified breads present increased weight, 720 ± 20 g and 718 ± 9 g, respectively for FBA and FBB samples, however, the increase was not statistically significant ($p>0.05$).

Concerning the bread volume mean SV of CBA samples (4.6 ± 0.3 cm^3/g) was similar to mean SV of FBA (4.5 ± 0.2 cm^3/g), whereas the mean SV of CBB was 3.9 ± 0.2 cm^3/g and FBB was 3.9 ± 0.1 cm^3/g. Two-way ANOVA indicate that no significant differences were observed due to β-glucans fortification by dry spent yeast addition ($p>0.05$), but the machine type had a significant effect on the bread volume ($p<0.05$). The two paddle machine (A) promoted an increase of bread volume. According to Cauvain (188) improvement of bread volume is achieved with vigorous mixing and kneading.

Mean and standard deviation of crumb and crust texture and colour of control and fortified breads from two different types of machine are summarized in Table 2.2. Two-way ANOVA performed to evaluate the effect of β-glucans fortification by dry spent yeast addition and machine type revealed that in general no significant effects were observed on texture and colour parameters. Regarding the texture of control and fortified breads, only springiness had significant differences ($p < 0.05$) in the crumb and crust. With regard to colour, the L* values of crumb were significantly different in control and fortified breads ($p < 0.05$). Concerning the effect of machine type significant differences were observed in crust hardness ($p < 0.05$). In conclusion, fortification with β-glucans darkened the crumb and increased crumb and crust springiness, which are characteristics appreciated by consumers.

Table 2.2. Mean values and Standard Deviation (SD) of Crumb and Crust Texture and Colour parameters

		Mean*±SD			
		Crumb		Crust	
		CB	FB	CB	FB
Texture	Machine				
Hardness	A	1.13 ± 0.13	1.60 ± 0.25	3.12 ± 0.62†	3.58 ± 0.36†
	B	2.21 ± 0.30	2.39 ± 0.57	4.57 ± 0.71††	4.85 ± 1.08††
Cohesiveness	A	0.74 ± 0.04	0.76 ± 0.02	0.82 ± 0.02	0.84 ± 0.03
	B	0.76 ± 0.04	0.77 ± 0.03	0.84 ± 0.01	0.86 ± 0.02
Springiness	A	0.67 ± 0.06^{a}	1.68 ± 0.07^{b}	0.781 ± 0.02^{a}	1.56 ± 0.04^{b}
	B	0.69 ± 0.06^{a}	1.54 ± 0.02^{b}	0.81 ± 0.01^{a}	1.43 ± 0.02^{b}
Chewiness	A	0.48 ± 0.14	2.59 ± 0.83	5.24 ± 0.87	14.18 ± 2.68
	B	2.08 ± 1.74	5.55 ± 2.00	12.00 ± 4.34	26.14 ± 11.62
Colour					
L*	A	70.21 ± 2.33^{a}	72.41 ± 1.19^{b}	50.89 ± 9.32	46.64 ± 4.73
	B	69.39 ± 1.00^{a}	72.11 ± 1.68^{b}	51.39 ± 9.39	51.37 ± 7.64
a*	A	1.08 ± 1.52	19.63 ± 1.67	0.87 ± 1.75	28.78 ± 5.75
	B	19.87 ± 0.85	21.50 ± 0.88	32.10 ± 2.02	30.31 ± 4.08
b*	A	0.26 ± 0.32	0.06 ± 0.37	12.78 ± 3.84	15.09 ± 1.54
	B	0.28 ± 0.20	0.20 ± 0.33	15.24 ± 1.40	14.46 ± 5.8

a*, redness; b*, yellowness; L*, lightness.

Differences were tested according to two-way ANOVA. In a column for each parameter, different number of symbol † and †† indicates significant differences ($p < 0.05$) due to machine type. In a line, different letters (a and b) indicate significant differences ($p < 0.05$) in crumb or crust due to β-glucans fortification by dry spent yeast addition.

2.3.3. Changes in volatile profile and quantification of relevant aroma compounds

Concerning optimization of SPME parameters, the response evaluated during all experiments was the total sum of peak areas, obtained in the GC-MS analysis and the number of identified compounds. Conditions adopted were those that gave greater total peak area and higher number of volatile compounds. Table 2.3 presents means results obtained in the six tests. The SPME process is influenced by temperature because the partition coefficients are temperature dependent. In general, the number of compounds extracted increased with temperature and with the extraction time. According to results from Table 2.3 the HS-SPME conditions selected were 2 g of bread in a 50-mL vial containing 10 mL of a 20% NaCl solution (pH 3 with citric acid 0.05M), and extraction with CAR/PDMS fibre during 60 min at 50 ºC under constant agitation (600 rpm).

Table 2.3. Optimization of Extraction Conditions for HS-SPME

Test	Water volume (189)	Salt (g)	20% NaCl solution pH 3	temp	Time (min.)	Peak area x10^9*	Compounds Number**
1	-	-	-	37	40	9.01	41
2	-	3	-	37	40	10.01	37
3	5	3	-	37	40	11.56	49
4	5	3	-	50	40	11.88	53
5	5	3	-	50	60	11.01	56
6	-	-	10	50	60	12.74	61

*Expressed as arbitrary units of area, mean value of duplicate analyses; ** Number of compounds identified in the chromatograms.

The selected HS-SPME conditions were applied to the analyses of control and fortified breads. Figure 2.1.A shows the relative percentage of area volatile compounds grouped by chemical classes extracted from control and fortified breads. Tool bars present mean values obtained for 6 bread samples (3 from machine A and 3 from machine B). The bread volatile profile when expressed as relative percentage of area, shows that in control breads the major volatiles were alcohols, aldehydes, alkanes and aromatic compounds, whereas in fortified breads alkanes and aromatics were the predominant chemical classes. However, as shown in Figure 2.1.B the total area of volatiles in CB breads is significantly lower when compared with the total area of volatiles in FB ($p < 0.05$, t-test). Thus, a detailed analysis of volatile profile was performed using individual peak area.

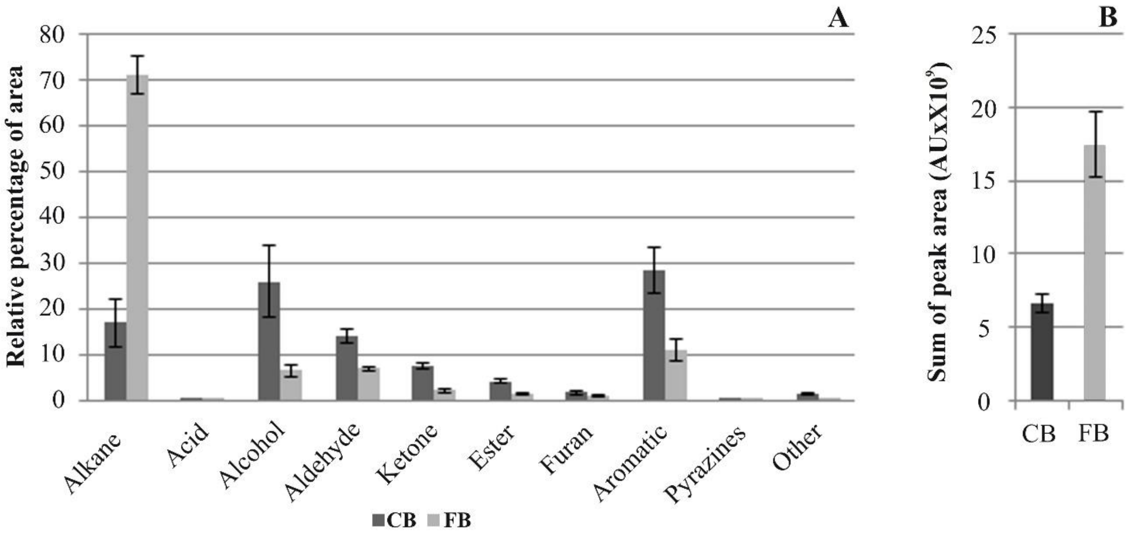

Figure 2.1. A) Relative percentage of area volatile compounds grouped by chemical classes extracted from control and fortified breads; **B)** mean total area of volatiles in control breads (CB) and fortified breads (FB).

Table 2.4 shows mean values of peak area of the volatile compounds identified in control (56 volatiles) and fortified breads (57 volatiles). Two-way ANOVA was performed using peak area of volatiles to evaluate the effect of β-glucans fortification by dry spent yeast addition and the effect of machine type. However, for 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-octen-3-ol, cis-4-decen-1-ol, 2-octanone, 2,6,8-trimethyl-4-nonanone, methyl acetate and styrene the significant interaction between yeast fortification and machine type was the most important effect, thus, two-way ANOVA was not performed on the main effects. Concerning the effect of fortification, the peak area of hexanal and benzaldehyde increased in fortified breads from the two machines, although it was not statistically significant. Almost all alkanes as well as pentyl propanoate, ethyl hexanoate, ethyl decanoate, acetylfuran and all pyrazines increased significantly the peak area in fortified breads. Only 4-methyl-2-pentanone decreased significantly in fortified breads. In fortified breads 2-ethyl-1-hexanol, 1-octanol, 3-pentanone and ethyl acetate were not detected although they were present in control breads, whereas 2-methyl-1-hepten-6-one was only detected in fortified breads. The effect of bread machine was less prominent, since significant differences were observed only in 2-phenylethanol, *trans*-2-nonenal, octanoic acid, ethyl octanoate, furfural, acetylfuran, and 2,6-dimethylpyrazine.

Table 2.4. Means values of peak area of volatiles in control and fortified breads. The results of two-way ANOVA are shown for the two factors: yeast amount (ys) and machine type (mach) and their possible interaction (ys*mach)

Compounds	**Odour**	**Mean values of peak area x 10^{7} (n=3)**				**ys*mach^{a}**	**Two-way ANOVA^{d}**	
		CBA	FBA	CBB	FBB	*p*-value	ys (*p*-value)	mach (*p*-value)
Alcohols								
Ethanol^{b}	alcohol^{d}	30.2	31.3	57.0	45.2	ns	ns	ns
2-Methyl-1-propanol^{b}	wine-like^{d}	2.41^{b}	nd^{a}	5.64^{c}	nd^{a}	<0.001	-	-
3-Methyl-1-butanol^{b}	fruity, sweet^{e}	28.41^{ab}	24.88^{a}	70.77^{b}	18.32^{a}	0.030	-	-
2-Methyl-1-butanol^{b}	cooked, roasted, fruity, alcohol^{e}	6.70^{ab}	7.01a^{b}	20.28^{b}	4.29^{a}	0.032	-	-
1-Hexanol^{b}	woody, sweet, green, fruity^{e}	42.27	37.90	53.83	39.3^{4}	ns	ns	ns
1-Octen-3-ol^{c}	sweet, earthy, herbaceous^{e}	1.90^{b}	nd^{a}	2.65^{c}	nd^{a}	0.017	-	-
2-Ethyl-1-hexanol^{c}	oily, sweet, floral^{e}	1.80	nd	1.67	nd	ns	0.002	ns
1-Octanol^{b}	fresh, orange-rose^{e}	2.07	nd	2.49	nd	ns	0.001	ns
2-Phenylethanol^{b}	rose^{e}	6.68	8.72	17.33	15.74	ns	ns	0.004
cis-3-Nonen-1-ol^{c}	fresh, waxy, green, melon^{e}	4.35	4.07	5.85	6.35	ns	ns	ns
cis-4-Decen-1-ol^{c}	waxy, fruity^{e}	nd^{a}	0.84^{a}	4.09^{b}	4.01^{b}	0.047	-	-
Aldehydes								
2-Butenal^{c}		nd	0.59	2.25	0.54	ns	ns	ns
3-Methyl-butanal^{b}	fruity, fatty^{e}	3.36	2.97	3.43	2.91	ns	ns	ns
2-Methyl-butanal^{b}	fruity, sweet^{f}	1.24	1.53	16.88	1.80	ns	ns	ns
Hexanal^{b}	fatty, green, grassy, fruity^{e}	23.73	35.13	26.69	30.54	ns	ns	ns
Heptanal^{b}	fatty, pungent^{e}	18.90	19.49	11.84	24.78	ns	ns	ns
trans-2-Heptenal^{c}	pungent, green, fatty^{e}	1.28	16.99	15.52	2.33	ns	ns	ns
Benzaldehyde^{b}	almond^{e}	22.08	36.55	16.07	31.70	ns	ns	ns
Octanal^{c}	fatty, citrus, honey^{e}	4.95	3.26	2.30	3.33	ns	ns	ns
trans-2-Octenal^{c}	green-leafy^{e}	1.43	2.18	1.96	2.74	ns	ns	ns
Nonanal^{c}	fatty, orange, rose^{e}	2.18	2.48	3.16	3.18	ns	ns	ns
trans-2-Nonenal^{b}	fatty, orris-like, waxy, orange^{e}	3.51	4.17	3.08	4.11	ns	ns	0.049
Decanal^{c}	sweet, waxy, floral, citrus, fatty^{e}	1.48	1.69	2.00	3.29	ns	ns	ns
Alkane								
Pentane^{b}		1.05	73.11	nd	54.13	ns	0.008	ns
2,2-Dimethylbutane^{c}		nd	3.17	nd	3.93	ns	0.007	ns
2,3-Dimethylbutane^{c}		nd	341.21	nd	25.49	ns	ns	ns
2-Methylpentane^{c}		46.21	534.16	31.31	573.14	ns	0.001	ns
Hexane^{b}		83.21	431.70	50.35	426.09	ns	<0.001	ns
Propylcyclopropane^{c}		nd	4.00	nd	4.24	ns	<0.001	ns
1-Methoxypentane^{c}		2.36	1.16	2.23	1.29	ns	0.042	ns
Decane^{c}		2.53	1.58	2.11	1.45	ns	ns	ns

Table 2.4. Means values of peak area of volatiles in control and fortified breads. The results of two-way ANOVA are shown for the two factors: yeast amount (ys) and machine type (mach) and their possible interaction (ys*mach) (Continued)

Compounds	**Odour**	**Mean values of peak area x 10^7 (n=3)**				**ys*mach[a]**	**Two-way ANOVA[d]**	
		CBA	FBA	CBB	FBB	*p*-value	ys (*p*-value)	mach (*p*-value)
Ketone								
2-Propanone[b]	fruity, apple, cooked meat[g]	nd	0.77	nd	10.50	ns	ns	ns
2-Butanone[b]	sweet, apricot[e]	1.73	nd	1.65	nd	ns	ns	ns
2-Pentanone[c]	ethereal, fruity[e]	17.24	11.00	14.96	7.69	ns	ns	ns
3-Pentanone[c]		8.18	nd	8.16	nd	ns	0.001	ns
3-Hydroxy-2-butanone[c]	woody, yogurt[e]	2.04	4.01	1.50	1.80	ns	ns	ns
4-Methyl-2-pentanone[c]	fruity, ethereal, spicy[e]	13.62	7.97	13.15	5.86	ns	0.004	ns
2-Methyl-1-hepten-6-one[c]		nd	2.84	nd	0.70	ns	0.046	ns
6-Methyl-5-hepten-2-one[c]	fatty, green, citrus[e]	2.23	2.23	2.93	2.93	ns	ns	ns
2-Heptanone[c]	fruity, spicy[e]	3.05	2.76	3.18	3.88	ns	ns	ns
2-Octanone[c]	floral, bitter, green, fruity[e]	2.00	2.75	2.04	2.32	ns	ns	ns
2,6,8-Trimethyl-4-nonanone[c]		0.37^{b}	nd^{a}	nd^{a}	nd^{a}	0.007	-	-
Acids								
Octanoic acid[b]	fruity, acid[e]	0.63	1.21	1.58	2.52	ns	ns	0.041
Ester								
Methyl acetate [c]	Fruity[e]	0.70^{b}	nd^{a}	nd^{a}	nd^{a}	0.029	-	-
Ethyl acetate[b]	ethereal fruity, brandy[e]	2.88	nd	2.78	nd	ns	<0.001	ns
Dimethyl carbonate[c]		1.12	nd	0.72	nd	ns	ns	ns
Methyl propanoate[c]		2.86	2.78	2.71	2.78	ns	ns	ns
Methyl butyrate[b]	apple[e]	18.95	12.90	16.12	8.07	ns	ns	ns
Pentyl propanoate[c]	fruity, rum[e]	nd	2.97	nd	2.43	ns	0.018	ns
Ethyl hexanoate[c]	fruity[e]	nd	0.44	nd	0.42	ns	0.008	ns
Ethyl octanoate[c]	fruity, floral[e]	0.99	1.67	2.71	2.56	ns	ns	0.015
Ethyl decanoate[c]	fruity[e]	1.23	3.05	1.19	2.81	ns	<0.001	ns
Aromatics								
Toluene[b]	benzene-like[h]	165.55	147.15	133.71	162.77	ns	ns	ns
Phenylacetaldehyde[c]	green[e]	2.67	1.60	1.50	2.04	ns	ns	ns
Chlorobenzene[c]		16.13	10.45	13.67	10.62	ns	ns	ns
o-Xylene[c]		0.55	1.12	0.83	0.88	ns	ns	ns
Methoxy-phenyl-oxime[c]		0.75	nd	nd	nd	ns	ns	ns
Styrene[c]	sweet, balsamic[e]	5.47^{b}	3.49^{a}	4.89^{a}	3.97^{a}	0.015	-	-
m-Xylene[c]		3.31	3.53	2.59	3.40	ns	ns	ns
1,2,3-Trimethylbenzene[c]		0.68	nd	0.63	nd	ns	ns	ns
Acetophenone[c]	sweet, pungent, medicinal[e]	165.55	147.15	133.71	162.77	ns	<0.001	ns

Table 2.4. Means values of peak area of volatiles in control and fortified breads. The results of two-way ANOVA are shown for the two factors: yeast amount (ys) and machine type (mach) and their possible interaction (ys*mach) (Continued)

Compounds	**Odour**	**Mean values of peak area x 10^7 (n=3)**				**ys*mach**[a]	**Two-way ANOVA**[d]	
		CBA	FBA	CBB	FBB	*p*-value	ys (*p*-value)	mach (*p*-value)
Furans								
Furfural[b]	fresh baked bread[i]	2.84	4.30	5.91	6.47	ns	ns	0.002
Furfuryl alcohol[b]	ethereal, floral, fruity[e]	2.75	5.97	4.87	6.60	ns	ns	ns
Acetylfuran[b]	coffee-like[e]	nd	0.10	0.23	0.36	ns	0.006	<0.001
2-Pentylfuran[b]	fruity[e]	2.17	4.99	3.32	3.53	ns	ns	ns
Pyrazines								
2,6-Dimethyl-pyrazine[c]	cooked rice, sweet[j]	nd	0.19	0.23	0.46	ns	<0.001	<0.001
3-Ethyl-2,5-dimethylpyrazine[c]		nd	0.52	nd	1.04	ns	0.006	ns
2,5-Diethyl-3-methylpyrazine[c]		nd	0.63	nd	1.06	ns	0.024	ns
Others								
Tetrachloroethylene[c]		8.71	7.61	7.77	5.94	ns	ns	ns

CBA, Control bread machine A; CBB, Control bread machine B; FBA, Fortified bread machine A; FBB, Fortified bread machine B; nd, not detected; ns, not significant

[a] If a significant interaction effect was found, the two-way ANOVA is not performed on the main effects and one-way ANOVA is used instead; yeast amount and machine separately, hence the interaction effect is then the most important effect.

[b] Identification obtained by comparing authentic standards with their mass spectra.

[c] Identified by NIST 05.

[d] Two-way ANOVA performed on the main affects

[e] Burdock (190)

[f] Mahattanatawee *et al.* (191)

[g] Garcia-Gonzalez *et al.* (192)

[h] Gu *et al.* (193)

[i] Fahlbusch *et al.* (194)

[j] Pham *et al.* (195)

Mean and standard deviation values of the quantification of key odour compounds from bread are presented in Table 2.5. For each compound the ion with greatest abundance was used for quantification purposes. The most abundant key odour was 3-mehyl-1-butanol and *trans*-2-nonenal was the key odour found in lower quantity. In general, the results from quantification by external standard method using an internal standard and expressed as µg/kg are in agreement with peak area quantification of volatile profile, although the quantification with calibration curves is more reliable as described by Petisca *et al.* (184). Two-way ANOVA indicates that bread fortification increased hexanal content, while the type of machine influenced 2-phenylethanol content. Quantification of crumb volatiles in bread samples prepared with different yeast concentration and fermentation temperature and also in bread prepared with different types of baker's yeast was performed by dynamic headspace (175, 176). The most abundant volatile was 3-mehyl-1-butanol, which is in agreement with our results, however quantitative comparison of this and other key volatiles with literature is difficult because only crumb was analysed and not the whole bread and also due to the high variability of results, depending on bread making and experimental analytical conditions and time between bread preparation and volatile analyses.

Analyses of volatile profile of control and fortified breads and quantification of key odour compounds indicates that β-glucans fortification by dry spent yeast addition has impact on volatile profile, although concerning key odours only hexanal presented a significant increase in fortified bread. However, hexanal is an aroma active compound that is often characterized as off-flavour (175, 176).

Table 2.5. Mean values (µg/kg) and Standard Deviation (SD) of the Volatile Compounds Quantified

Compound	**Ion m/z**	**Mean ± SD**				ys*mach	Two-way ANOVA	
		CBA	**FBA**	**CBB**	**FBB**	*p*-value	ys (*p*-value)	mach (*p*-value)
3-Methylbutanal	44	12.1 ± 3.3	10.4 ± 1.0	9.7 ± 6.5	12.7 ± 1.7	ns	ns	ns
3-Methyl-1-butanol	55	563 ± 22	477 ± 125	813 ± 140	430 ± 14	ns	ns	ns
2-Phenylethanol	91	8.1 ± 0.2	8.8 ± 0.5	10.8 ± 0.5	13.1 ± 1.7	ns	ns	0.021
Hexanal	44	8.9 ± 0.1	13.6 ± 0.5	7.9 ± 1.6	13.9 ± 2.0	ns	0.014	ns
trans-2-Nonenal	43	0.04 ± nd	0.04± 0.01	0.04 ± 0.01	0.09 ± 0.02	ns	ns	ns

CBA, Control bread machine A; CBB, Control bread machine B; FBA, Fortified bread machine A; FBB, Fortified bread machine B; ns, not significant

If a significant interaction effect was found, the two-way ANOVA is not performed on the main effects and one-way ANOVA is used instead; yeast amount and machine separately, hence the interaction effect is then the most important effect.

2.3.4. Impact of β-glucans fortification by dry spent yeast addition on sensory characteristics of bread

Figure 2.2 summarizes results from sensory analyses in a spider chart. The general profile of control and fortified breads from the two machines is similar. Two-way ANOVA was performed to evaluate the effect of β-glucans fortification by dry spent yeast addition and the effect of machine type. For crumb colour, bread odour, bread aroma, and crispy crust the significant interaction between yeast fortification and machine type was the most important effect, thus, two-way ANOVA was not performed on the main effects. CBA samples presented significantly lower crumb colour, CBB presented significantly lower bread odour and crispy crust. No significant differences were found on bread aroma. Main effects of β-glucans fortification indicate significant differences on crust colour and odour intensity, although no significant differences were observed in the other attributes, namely, for overall assessment.

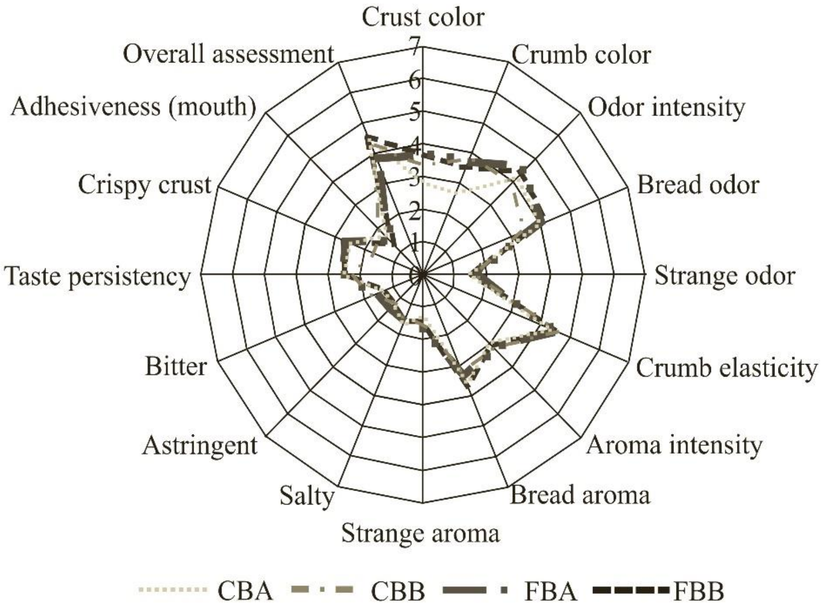

Figure 2.2. Spider chart representation of bread sensory characteristics

CBA, Control bread machine A; CBB, Control bread machine B; FBA, Fortified bread machine A; FBB, Fortified bread machine B

2.4. Conclusions

A serving of bread fortified with dry spent yeast increases β-glucans intake from 65 mg to 125 mg, a content within the guidelines suggested by EFSA. Although, this fortification darkened the crumb, increased crumb and crust springiness and had impact on volatile profile, concerning key odours (3-methylbutanal, 3-methyl-1-butanol, 2-phenylethanol, hexanal, and *trans* 2-nonenal) only hexanal presented a significant increase in fortified bread. Descriptive analyses performed by a trained panel corroborate differences on crust colour and odour intensity, although no significant differences were observed in the other attributes, namely, for overall assessment. Additionally, the machine type (with 1 or 2 paddles) has a minor impact on bread characteristics. Thus, dry spent yeast can be used as ingredient in bread to increase β-glucans intake without compromising the sensory characteristics, contributing to valorisation of by-products from the brewing industry. Moreover, the brewing yeast is recognized as a good source of chromium and B vitamins, thus further studies to quantify the increase of these bioactive compounds in fortified bread are pertinent.

CHAPTER 3

Search for new ingredients from brewer’s spent yeast to improve bread quality

Abstract

The impact of bread fortification with β-glucans rich extract and proteins/proteolytic enzymes from brewers' spent yeast on physical characteristics was evaluated. Moreover, bread with and without improver was also compared. β-Glucans extraction from spent yeast cell wall was optimized and the extract was incorporated on bread to obtain 2.02 g β-glucans/100 g flour, in order to comply with the European Food Safety Authority guidelines. Proteins/proteolytic enzymes from spent yeast were added to bread at 60 U proteolytic activity/100 g flour. β-Glucans rich extract and proteins/proteolytic enzymes from brewers' spent yeast influenced the crust and crumb colour in comparison with control bread and bread containing improver. Concerning the morphological features of crumb, significant differences among studied bread as were observed only for large size class (cell area > 10.0 mm2). Control and proteins/proteolytic enzymes fortified bread had the highest percentage of cells pertaining to this class, which indicates greater gas cells coalescence with coarser bread crumb structure. Therefore, only β-glucan rich extract seems a promising functional ingredient to improve bread technological and health promoting properties, since proteolytic enzymes extract had a negative impact on bread physical properties and quality.

Keywords: bread, β-glucans, proteins, proteolytic enzymes, brewing by-products

3.1. Introduction

The bread type most consumed in Portugal is a small round wheat bread with light airy texture and easy digestion. The light airy texture is obtained by the use of flour of good quality that is, flour with enough protein, particularly gluten, capable of forming dough of satisfactory elasticity, strength and stability. Moreover, improving agents are additives (improvers) used in bread making, including mixtures of oxidants, enzymes, emulsifiers and hydrocolloids that are usually added to flour to simplify the production and improve the quality of baking products (196, 197). Functional properties considered for bread improvers are mainly related with technological aspects. However, finding new functional ingredients for bread making that can improve both technological and nutritional/health properties is a challenging task (34).

Brewing spent yeast, the second major by-product from brewing industry can be rich source of functional ingredients, such as fibre (mainly β-glucans), vitamins, minerals, and protein including proteolytic enzymes and others (24-29).

Yeast β-glucans are natural cell wall polysaccharides and also a major structural component. Long chain (β-(1→3) and β-(1→6) linked glucose polymers), water-insoluble and nondigestible yeast β-glucans are able to modulate mucosal immunity of the intestinal tract, facilitates bowel motility and can be used in obstipation, among other intestinal problems (24, 159-161). The European Food Safety Authority (EFSA) has already approved the use of *Saccharomyces* β-glucans – referred to as “yeast beta-glucans” - as a new ingredient and suggests a use ranging between 50 and 200 mg of “yeast beta-glucans” per serving (165). Nerveless, the impact of “yeast beta-glucans” addition on bread texture is not known.

Yeast proteins and proteolytic enzymes are considered a GRAS and present an adequate amino acid profile rich in essential amino acids (25, 198-203). The use of exogenous enzymes in the baking industry is described to improve dough and bread quality and shelf life (199). However, addition of proteolytic enzymes to bread can also have a negative impact on baking quality. No studies were found concerning the effect of spent yeast protein/enzymes incorporation on bread light airy texture.

The objective of this work was to evaluate the impact of bread fortification with β-glucan rich extract and proteolytic enzymes from brewers’ spent yeast on bread physical properties and quality.

3.2. Material and methods

3.2.1. β-Glucan and proteolytic enzymes extraction from spent yeast

The spent yeast biomass obtained from Lager beer production (*Saccharomyces pastorianus*) was supplied by a local beer industry. β-Glucans rich extract was obtained from spent yeast through autolysis, alkaline extraction, and acid extraction, as described by Thammakiti *et al.* (204). Briefly, spent brewer's yeast suspension was adjusted for 15% w/w solid content and pH 5. Autolysis was carried out at 50 °C for 24 h with moderate agitation. Afterwards, the autolysate was heated at 80 °C for 15 min, cooled to room temperature and centrifuged at 3600 *g* for 10 min at room temperature. The pellet was suspended in distilled water and adjusted to 15% w/w solid content. Alkaline extraction was done using 1.0 N NaOH at 80 ± 5 °C at a ratio of 1:6 (v/v) for 2 h followed by an acid extraction with 0.5 N CH_3COOH at 75 ± 5 °C at a ratio of 1:6 (v/v) for 1 h. The mixture was centrifuged at 3600 *g* for 10 min at room temperature and then washed three times with distilled water. The obtained β-glucans rich extract was lyophilized and later milled with a Knife Mill Grindomix GM200 (Retsch GmbH, Germany). β-Glucans content was quantified using the “Enzymatic yeast beta-glucan – assay procedure” (Megazyme International Ireland Ltd., Bray, Ireland).

For the extraction of protein/proteolytic enzymes from spent yeast, the mechanic disruption process described by Vieira *et al.* (198) was adopted. The resulting proteolytic enzymes extract was lyophilized. Proteolytic activity was determined according to Mäkinen and Arendt (205).

3.2.2. Bread samples

Bread samples were produced at a pilot scale in an experimental laboratory (Ceres, Porto, Portugal). Wheat flour, commercial flour improver, fresh yeast, and salt, supplied by Ceres (Porto, Portugal) were used to carry out this study. Control bread (BC), breads with improver (BI) and breads fortified with β-Glucans (BβG) and proteins/proteolytic enzymes (BPT) were prepared following the recipes shown in Table 3.1. EFSA maximum recommendations on yeast β-glucans (200 mg/dose) were considered for the incorporation of β-glucans rich extract. For incorporation of proteins/proteolytic enzymes extract, maximum values used by Mäkinen and Arendt (205) were considered for the incorporation, i.e. 60 U proteolytic activity (mg leucine/h/g). Bread ingredients were mixed and kneaded for 20 min. After kneading, each 80 g of dough was shaped into a ball and proofed for 90 min at 30 °C with 80% relative humidity. Baking was performed for 10 min at 200 ºC with. Bread samples were cooled 90 min at room temperature before further analysis. Baking trials were conducted with six replicates.

Table 3.1. Recipe for each bread formulation

Ingredients (g)	Bread formulations			
	CB	**BI**	**BβG**	**BPT**
Wheat flour	1500	1500	1500	1500
Salt	30	30	30	30
Yeast	75	75	75	75
Improver	-	15	-	-
Water	900	900	900	900
β-Glucans rich extract	-	-	30.7	-
Proteolytic enzymes extract	-	-	-	4.7

CB, control bread; BβG, bread fortified with β-glucans; BI, bread with improver; BPT, bread fortified with proteins/proteolytic enzymes (BPT).

3.2.3. Evaluation of bread physical characteristics

3.2.3.1. Weight, specific volume, and moisture

Breads weight and specific volume were measured 90 min after removing from oven (n = 6). The bread specific volume (SV) was measured using a seed displacement method (Cerealis internal method) and the following formula

$$SV = Sx1.35/P \tag{3.1}$$

P (g) bread weight; S (g) Weight of the displaced seeds, 1.35 (cm^3/g) Specific volume of the *Phalaris canariensis* seeds, SV (cm^3/g) Specific volume of the bread.

Bread moisture was assayed by infrared drying at 105 °C (Scaltec SMO 01, Heiligenstadt, Germany) until constant weight and expressed as g of moisture per 100 g of bread.

3.2.3.2. Crumb structure image analysis

To study the crumb structure, breads of every formulation were cut (1.6 cm thickness) and analysed. Each slice was positioned on the flatbed scanner (Canon iR2016i, Netherlands) in pre-standardized conditions (black cardboard box over the slice, in order enhance contrast) (206). Images were captured in the RGB (24 bit) standard format with a resolution of 300 dpi and saved in JPG format. Each image was processed and analysed using Matlab R2015a (MathWorks). A single 200x200 pixel (85x85 mm) field of view (FOV) was cropped from the image centre and converted to a 256 level grey scale. Image segmentation was performed with Otsu's method (207) and cell morphological parameters were analysed with Image Processing Toolbox 9.4 from Matlab. Data resulting from the crumb structure analysis included: number of cells, mean cell area (mm^2), and cell density (cells/mm^2). Additionally, cells were separated into different classes as a function of their area: very small size (0.2 mm^2 ≤ cell area); small size (0.2 < cell area ≤ 3.0 mm^2); medium size (3.0 < cell area ≤ 10.0 mm^2); large size (cell area > 10.0 mm^2).

3.2.3.3. Evaluation of bread colour

For colour analysis, six bread of every formulation were cut in half and the measurements were performed in three different points on the crumb and side crust.

Colour measurement was done in the CIElab system – lightness (L*), redness (a*) and yellowness (b*). A Minolta CR-300 colorimeter (Minolta, Ramsey, NJ) with illuminate D65, a 0° standard observer and a 2.5 cm port/viewing area was used and standardized with a white tile having the following values: L* = 93.5, a* = 1.0 and b* = 0.8 before measurement in crust and crumb..

3.2.4. Statistical analysis

All dependent variables from every BB formulation analysed were tested for the residuals distribution with Shapiro–Wilk's test. Improver addition, as well as β-glucans rich extract and proteins/proteolytic enzymes extract incorporation were studied using a one-way ANOVA or Kruskal–Wallis test, according to the residuals distribution. Whenever statistical significances were found with one-way ANOVA, Tukey´s or Tamhane's T2 post-hoc tests were applied for mean comparison, depending on equal variances assumption or not. When statistical significances were found with Kruskal–Wallis test, Dunn's test post-hoc test was applied for median comparison. All statistical analyses were conducted with the XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France) at 5% significance level.

3.3. Results and discussion

3.3.1. Bread weight, specific volume, and moisture

As seen in Table 3.2, weight and moisture did not vary significantly the different bread formulations. As for specific volume, significant differences were found between BI and BC, BβG, and BPT, which highlights the improver importance in bread production. According to Pomeranz *et al.* (122), a reduction in specific volume occurs by dilution of the gluten content and changes in crumb structure, which in turns impairs carbon dioxide retention. While the highest specific volume was observed for BI, the lowest was for BPT. Chen *et al.* (123) suggested that fibre molecules may perhaps interact with wheat flour proteins and interfere with gluten development. Additionally, no significant differences were registered between BC and BβG.

Table 3.2. Values for physical parameters, colour, and crumb image analysis for BB with different formulations

Physical parameters	Bread Sample				*p*
	CB	BI	BβG	BPT	
Weight (g)	47.1 ± 1.5	44.6 ± 1.6	45.0 ± 1.0	47.0 ± 1.8	ns
Specific volume (cm^3/g)	3.0 ± 0.2^{b}	7.1 ± 0.5^{a}	3.2 ± 0.2^{b}	2.3 ± 0.1^{c}	<0.001**
Moisture (%)	28.87 ± 1.25	29.40 ± 1.73	24.62 ± 3.27	26.51 ± 1.94	ns
Crust					
L*	71.62^{b} (60.83 – 75.32)	66.66^{a} (42.87 – 78.14)	71.01^{b} (62.89 – 76.31)	66.37^{a} (58.22 – 71.19)	<0.001***
a*	3.23 ± 1.17^{b}	6.92 ± 3.67^{a}	4.54 ± 1.47^{c}	5.27 ± 1.55^{c}	<0.001**
b*	17.35^{b} (32.44 – 24.90)	12.70^{a} (40.09 – 33.57)	20.65^{ac} (33.32 – 27.51)	21.21^{bc} (31.22 – 26.80)	<0.001***
Crumb					
L*	61.91 ± 3.34^{b}	68.60 ± 3.57^{a}	64.85 ± 2.84^{c}	57.72 ± 3.23^{d}	<0.001*
a*	0.56^{bc} (0.08 – 1.06)	-1.02^{a} (-1.26 – -0.64)	0.67^{b} (0.20 – 1.08)	0.41^{c} (0.06 – 1.06)	<0.001***
b*	15.45 ± 0.72^{b}	11.74 ± 1.58^{a}	17.98 ± 0.64^{c}	15.95 ± 0.64^{d}	<0.001**
Number of cells	380 ± 50	395 ± 40	384 ± 80	373 ± 43	ns
Mean area (mm^2)	9.87 ± 0.88	10.06 ± 1.43	12.29 ± 2.51	9.69 ± 0.55	ns
Cell density (cells/mm^2)	38.69 ± 6.01	39.83 ± 6.36	40.17 ± 14.88	37.41 ± 8.05	ns
Cell area (% of total cells)					
0.2 ≤ CA (mm^2)	41.02 ± 2.34	42.35 ± 3.06	43.99 ± 0.37	39.85 ± 2.07	ns
0.2 < CA ≤ 3.0 (mm^2)	44.41 ± 1.89	45.12 ± 1.94	43.62 ± 2.29	45.06 ± 2.31	ns
3.0 < CA ≤ 10.0 (mm^2)	7.01 ± 1.81	7.02 ± 1.45	6.22 ± 1.57	7.56 ± 0.99	ns
CA > 10.0 (mm^2)	8.00 ± 0.32^{b}	5.51 ± 1.21^{a}	6.42 ± 2.65^{ab}	7.97 ± 0.35^{b}	0.006**

CA, cell area; CB, control bread; BβG, bread fortified with β-glucans; BI, bread with improver; BPT, bread fortified with proteins/proteolytic enzymes; ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution

$*$ *p* Values from one-way ANOVA analysis. Means were compared by Tukey’s test, since homogeneity of variances was confirmed by Levene’s test ($p > 0.05$).

$**$ *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

$***$ *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

3.3.2. Bread colour and morphological features

The effect of improver addition and bread fortification with β-glucans or with proteolytic enzymes on appearance and crumb structure was illustrated in Figure 3.1. Indeed the appearance of BC and BI differed significantly, since BI presented higher volume, height and darker colour. β-Glucans and proteins/proteolytic enzymes fortification had a different impact on appearance and crumb structure when compared with BC. BβG presented high height and a golden colour than BC and BPT. In BPT, the characteristic crust mid-section crack is not present.

Instrumental analysis indicate that crust and crumb colour of different bread formulations varied considerably (Table 3.2). Accordingly with illustrated in Figure 3.1, significant differences ($p < 0.05$) were found between BC and BI for every colour parameter.

In relation to bread crust colour parameters, crust L* was significantly lower (darker) for BI and BPT than for other formulations. Besides, no significant differences were detected between BC and BβG. Bread crust exhibited a reddish colour (positive a*) for every bread formulation, with BI presenting the highest values. BβG and BPT crust a* values were significantly higher than BC. Crust b* was positive for every bread formulations that exhibited a yellowish colour. The highest b* values were found for BβG and the lowest for BC.

Regarding crumb L*, BPT presented the lowest values (darker) while BI the highest. Also, significant differences were observed between BC, BβG, and BPT. A decrease in crumb L* was also observed by Martins *et al.* (127) with the addition of extract recovered from brewing spent yeast. While BI had a greenish crumb colour (negative a* values), BC, BβG, and BPT showed a reddish crumb colour (positive a* values). BβG and BPT crumb a* values were significantly higher than CB. Crumb b* values were positive for every BB formulations (yellowish colour). The highest b* values were observed with BβG and the lowest with BI. Moreover, significant differences were found between CB, BβG, and BPT. Bread colour depends on factors such as formulation or baking conditions. The Maillard reaction and caramelisation in crust is responsible for the brown colour formation, but it can be masked by colour changes attributed to different ingredients used in bread formulation (134, 135).

Crumb morphological features of the different bread formulations are compared in Table 3.2. Crumb morphology is an important bread quality parameter alongside taste, crumb colour and crumb texture (208, 209). Regarding number of cells, mean area, and cell density, only minor variations were observed. When observing cell area distribution, more than 84% of cells in every bread formulation had an area below 3.0 mm^2. Nevertheless, significant differences in cell area distribution were only found for large size class (cell area > 10.0 mm^2). CB and BPT had the highest percentage of cells pertaining to this class, while BI had the lowest. Bread cells features depend on gas retention capacity of the gluten network, as well as on maintenance of gas cells integrity throughout fermentation and baking (209). The presence of some proteases, as described by Capocchi *et al.* (210) can result in the hydrolysis of gluten proteins decreasing dough strength. In order to gas cells conserve integrity, they need to be sufficiently extensible to allow expansion and strongh enough to prevent premature rupture (211-213). Otherwise, small gas cells will coalesce into larger ones. The greater the gas cells coalescence is, the coarser is the bread crumb structure, which in turn affects the bread quality negatively (214).

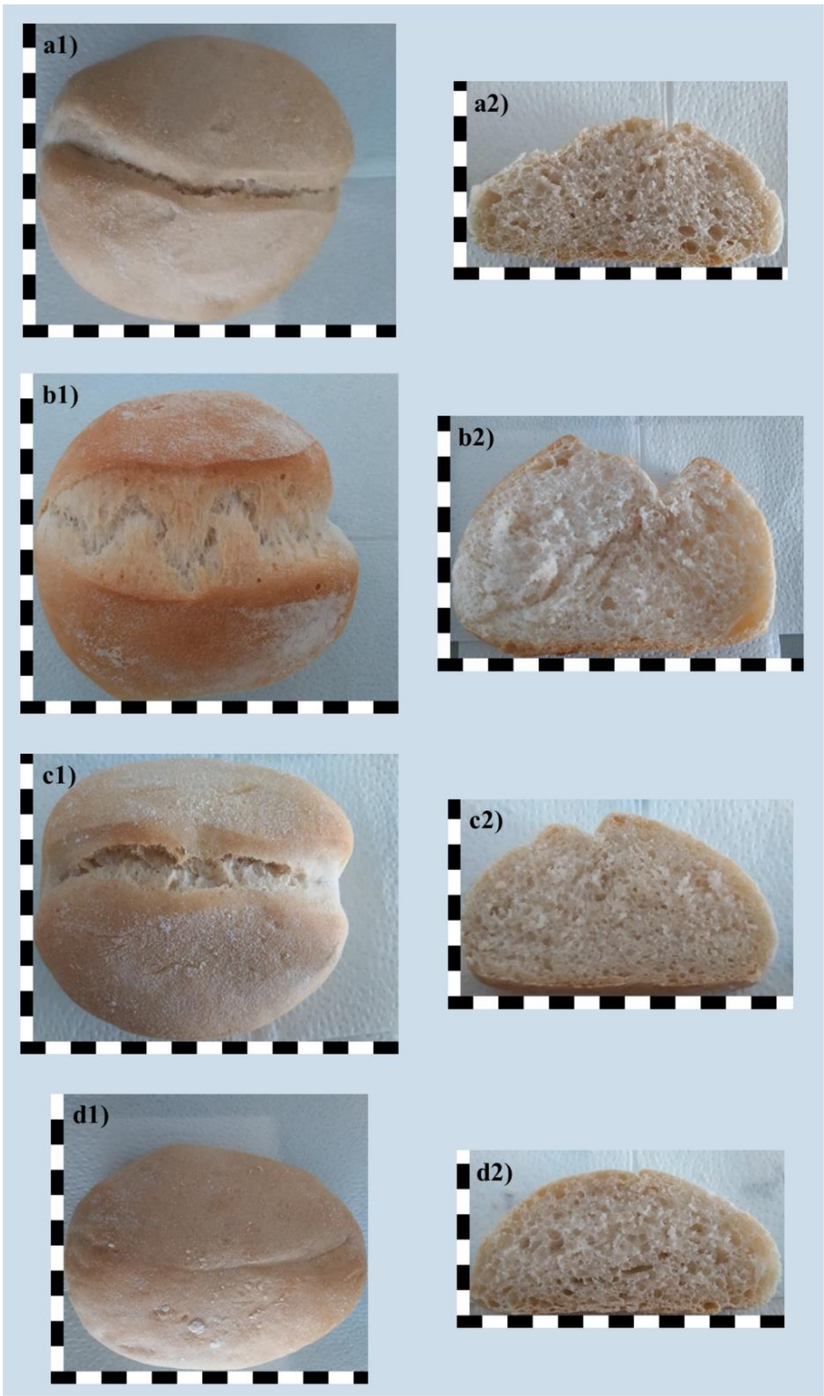

Figure 3.1. Overall observations (1) and cross-sections (2) of breads. a) Control bread (CB), b) bread with improver (BI), c) bread fortified with β-glucans (BβG), and d) bread fortified with proteins/proteolytic enzymes (BPT). One rectangle in the scale is 1 cm (width, height)

3.4. Conclusions

β-Glucans rich extract improved the technological performance of bread, compared to control bread. Additionally, it presented some similarities with bread containing the improver. In contrast, proteins/proteolytic enzymes extract had a negative impact on bread physical properties and quality. Therefore, β-glucans rich extract seems a promising functional ingredient to improve bread technological and health promoting properties.

PART III

AGROINDUSTRY BY-PRODUCTS IMPACT ON WHEAT DOUGH AND BREAD CHARACTERISTICS

CHAPTER 4

Development of fibre enriched wheat breads: Impact of recovered agroindustry by-products on physicochemical properties of dough and bread characteristics

Abstract

Dietary fibre is easy available in plant foods. However, western diet frequently does not meet recommended levels. Fibre fortification of bread is an opportunity due to its daily consumption. In this work, fibre enriched extracts were recovered from elderberry (EE), orange (OE), pomegranate (PE), and spent yeast (YE), and their fibre composition was characterized. The impact of wheat flour replacement by different fibre extract amounts on dough properties indicates that: i) optimum water absorption increased with higher concentrations of OE, PE, and YE; ii) development time for EE, PE, and YE was shortened while the opposite was observed for OE; iii) the onset of starch gelatinization and maximum tanδ significantly increased with 36% EE and 4% PE; iv) protein structure, observed with confocal laser scanning microscopy, was modified by addition of extracts; v) maximum and final dough height significantly decreased, except for 4% EE. Wheat flour replacement also had impact on bread parameters, since: i) volume and specific volume decreased at the highest concentrations in every extract; ii) significant changes were observed in crumb texture and structure, at higher extract concentrations. Multivariate PLS regression highlights relationships between dough and bread data.

Key words: source of fibre, high in fibre, dough analyses, bread analyses.

4.1. Introduction

Dietary fibre intake is generally accepted as having an important impact in human health (215-217). An adequate daily intake (25-30 g per day) has been associated with prevention or treatment of hypertension, cardiovascular diseases, diabetes, regulation of the intestinal tract and decrease in incidence of several types of cancer (140, 216, 218-220). Soluble dietary fibre (SDF), mainly pectins, cereal β-glucans, and some hemicelluloses contribute to reduced gastric emptying rates, glucose absorption, blood cholesterol and colon cancer protection whereas insoluble fibre (IDF) namely lignin, cellulose and some hemicelluloses have physiological functions, being able to decrease toxins and carcinogens contact with the intestinal tract (221). Although fibre is easily available in a wide variety of plant foods, western diet frequently presents an intake lower than the recommended (222). Thus, fibre fortification of foods consumed on a regular basis, such as bread is advisable.

Although bread consumption varies according with cultural habits, bread daily base intake makes it an interesting vehicle for dietary fibre (DF) incorporation (166, 168). DF addition to bread at levels required for the nutrition claims “source of fibre” and “high in fibre” is 3 and 6 g dietary fibre (DF)/100 g bread (fresh weight), respectively (13). Agroindustry by-products (BP) can be good sources of DF, which can be recovered and applied as food ingredient. This may support waste reduction and contribute to indirect income generation. Another interesting fibre source is spent yeast. This is the second major brewing BP and the cell walls are rich in yeast β-glucans (25, 223).

Several studies evaluated the influence of DF recovered from agorindustry BP in dough and bread formulations (53, 54, 117, 119). Recently, Sulieman *et al.* (19) analysed the influence of wheat bread enrichment with pomegranate peels BP (without any pre-treatment) at 5%. 7.5% and 10% . However, it affected strongly physicochemical properites and decreased organoleptic acceptance of final product. Moreover, Belghith Fendri *et al.* (36) evaluated extracted pea and broad bean pods fibers incorporation up to 1% in dough and bread. The addition of those fibers at 1% increased dough strenght and improved texture profile of enriched breads. However, studies related with the impact on dough fermentation characteristics, fundamental rheology, and microstructure of breads eligible as “source of fibre” and “high in fibre” remain an unexplored research area. Pineapple pomace fibre incorporation (5 and 10%) was evaluated by Chareonthaikij *et al.* (224) and, although affecting physicochemical properties dough and bread, 5% addition did not have a negative effect on consumer acceptance. At this level, bread TDF content was 4.4% and, although not being a goal, it could be ellegible for the nutrition claim “source of fibre”. According to several Food Databases, DF content of white wheat bread (the most consumed bread variety) ranges from 2.1 to 4.3 g/100 g bread (fw), whereas whole grain wheat bread varies between 4.0 and 8.2 g/100 g bread (fw) (225-227). Although some white wheat breads can be a “source of fibre” and some whole grain breads “high in fibre”, wheat bread fortification with fibre recovered from agroindustry BP can be used in order to guarantee the levels required for either nutrition claims.

In this work, fibre extracted from four types of agroindustry BP was used to prepare functional breads presenting the above mentioned claims and to understand the impact fibre addition on dough and bread physicochemical properties. For this propose, i) fibre enriched extracts were recovered from elderberry skin, pulp and seeds (EE), orange peel (OE), pomegranate peel and interior membranes (PE), and spent yeast (YE) and their fibre composition was characterized; ii) the impact of wheat flour replacement by the different fibre extract at various amounts was evaluated on dough (mechanical properties, microstructure and fermentation) and bread (volume, texture, and image analysis); iii) additionally, correlations between fibre addition, dough and bread characteristics were investigated through regression models.

4.2. Material and methods

4.2.1. Raw materials

The oranges and elderberries were supplied by local producers and the pomegranates were purchased in a market in Porto, Portugal. The spent yeast biomass used in Ale beer production (*Saccharomyces cerevisiae*) was supplied by the research brewery of the Technical University Munich, Freising, Germany.

German commercial wheat flour type 550 with 13.59 ± 0.12 g of moisture, 11.49 ± 0.04 g of protein, 3.87 g of dietary fibre (DF), and 0.65 ± 0.01 g of ash per 100 g of flour with a falling number of 437.33 ± 8.14 s was obtained from Rosenmühle (Landshut, Germany). Flour moisture was determined through flour sample heating at 130 ºC during 1 h. Flour protein, ash, and falling number were determined by ICC standard 105/7, ICC standard 104/1, and ICC standard 107/1, respectively. Dry yeast from the species *Saccharomyces cerevisiae* (Casteggio Liveti, Italy) was used for dough and bread formulations.

4.2.2. Extraction procedures and composition

Yeast extract (YE) was obtained from spent yeast through autolysis and then lyophilized, as described by (204) (Figure 4.1). The remaining by-products (Elderberry extract (EE); Orange extract (OE); Pomegranate extract (PE)) were subjected to different extraction procedures, summarized in Fig. 4.1.

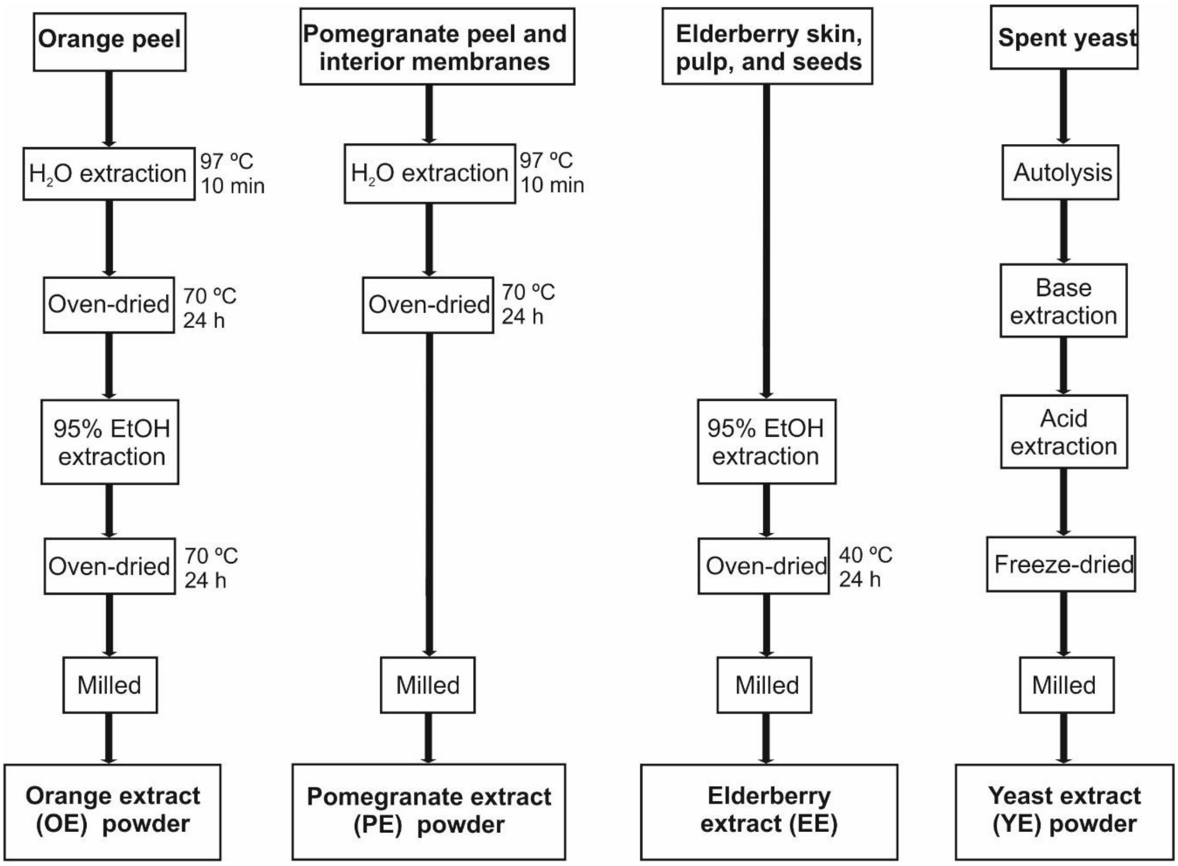

Figure 4.1. Extract preparation from different agroindustry by-products

Extract samples were characterised for their content in different dietary fibre (DF) fractions. Total Dietary Fibre (TDF) and Insoluble Dietary Fibre (IDF) contents were analysed, in triplicate, using a Megazyme Total Dietary Fibre kit, (K-TDFR, Megazyme International Ireland Ltd., Ireland), based on the methods of Prosky *et al.* (228) and Prosky *et al.* (229). A minor modification was done in order to optimize the recovery of the fibre residue (centrifugation prior to filtration). Soluble Dietary Fibre (SDF) content was determined as the difference between TDF and IDF values. TDF, IDF and SDF were also analysed for wheat flour.

(1-3)(1-4)-β-D-Glucan (1,3:1,4BG) was determined using a Beta-Glucan (Mixed Linkage) enzyme kit (K-BGLU, Megazyme International Ireland Ltd., Ireland), according with the assay procedure provided by the kit supplier. (1-3)(1-6)-β-D-Glucan and (1-3)-β-D-Glucans (1,3:1,6BG) content was determined using an Enzymatic Yeast β-Glucan kit (K-EBHLG, Megazyme International Ireland Ltd., Ireland), according with the assay procedure provided by the kit supplier.

Additionally, sodium and chlorine were determined by ion chromatography according to Pinto *et al.* (230) and flame atomic absorption spectrometry according to Pinto *et al.* (231), respectively.

4.2.3. Dough preparation

In order to comply with the nutrition claims “source of fibre” and “rich in fibre”, it was required to guarantee at least 4.08 and 8.16 g TDF/100 g flour mixture (dry basis), respectively. Eight kinds of bread were produced from different wheat flour replacement by extracts: 4% EE (flour mixture TDF content: 5.06%), 36% EE (14.62%), 4% OE (6.47%); 8% OE (9.07%); 4% PE (5.90%); 16% PE (12.35%); 4% YE (6.61%); and Control (3.71%). The optimum water absorption and kneading time (development time) were determined using a doughLAB with torque measuring Z-kneader and 50 g bowl (doughLAB; Perten Instruments, Germany), according to AACC method 54–70.01. All dough formulations were analysed in triplicate.

To reach 500 farino units, in the model dough (no extract replacing wheat flour), 59.0 g of distilled water/100 g flour, with moisture corrected to 14.00 g/100 g of flour, and a kneading time of 214 ± 77 s at 63 rpm and 30 °C were required. Optimum water absorption and development time were adjusted for doughs formulated with wheat flour replacement by extract, in order to reach 500 farino units.

4.2.4. Dynamic mechanical thermal analysis (DMTA) with oscillatory measurements

For the DMTA with oscillatory measurements an AR-G2 rheometer (TA instruments, New Castle, USA; software Rheology Advantage 5.7.2.0) with a Smart Swap Peltier plate temperature system and 40 mm plate-plate geometry (serrated surface) was used. Dough was prepared as described in the previous chapter and a portion was placed between the plates. After adjusting to the chosen gap (2 mm), the excess dough was removed with a spatula and the edges were sealed with paraffin, in order to prevent drying. The DMTA with oscillatory measurements were carried following the procedure of Jekle *et al.* (232), simulating the baking process from 30 ºC to 98 ºC using a temperature ramp of 4.25 ºC/min. Final data was expressed as loss factor tan δ and complex shear modulus $|G^*|$. Starch gelatinization temperature was determined as described by Jekle *et al.* (232). Data collected throughout temperature ramp for each bread formulation was analysed using Curve Fitting Toolbox 3.5.3 from Matlab R2016a (MathWorks, Natick, USA) and equations with best fitting performance (higher R^2 and lower RMSE) for complex shear modulus ($|G^*|$) and loss factor tan δ (tan δ) were selected. For $|G^*|$ equations ($f(x)$), the first ($f'(x)$) and second ($f''(x)$) derivatives were determined. While $f'(x) = 0$ was found in order to establish maximum $|G^*|$ ($|G^*|_{max}$) and respective temperature, the point where $f''(x) > 0$ was used to establish the value for significant increase of $|G^*|$ ($|G^*|_{onset}$) and respective temperature. For tan δ equations (g(x)), the first (g'(x)) derivative was determined and g'(x) = 0 was found in order to establish the maximum of loss factor tan δ (tan δ_{max}) and respective temperature. Every dough formulation was analysed in triplicate.

4.2.5. Protein microstructure analysis

For the protein microstructure analysis, Confocal Laser Scanning Microscopy (CLSM) micrographs were captured using an e-C1plus confocal system with a Ti-U inverted research microscope (Nikon, Düsseldorf; Germany) with a 20 x and 60x objectives. Dough was prepared as described previously (section *Dough preparation*), with addition of rhodamine B (Sigma-Aldrich Chemie Gmbh, Munich, Germany) in two concentrations (0.002 g/100 mL water and 0.004 mg/100 mL water). A portion of each dough was transferred afterwards to a specimen shape and covered with a glass slip. For dough formulations with EE replacement, rhodamine B had to be replaced by nile blue, as changes in the dough pH destroyed rhodamine B. Therefore, rhodamine B was substituted with 15 µL of nile blue (Sigma-Aldrich Chemie Gmbh, Munich, Germany) solution (0.1 g/ 100 mL water), which was added on top of the dough sample. Each dough formulation was prepared in triplicate and ten different micrographs were acquired for each objective and every dough sample in independent positions on the xy-axis. Proteins were monitored through fluorescence with $\lambda_{excitation}$ = 543.5 nm and $\lambda_{emission}$ = 590 nm (rhodamine B), and with $\lambda_{excitation}$ = 488.0, 543.5, 632.8 nm and $\lambda_{emission}$ = 590 nm (nile blue). Each micrograph viewed with 20x objective covered an area of 686x686 µm, and 212x212 µm with 60x objective, at constant z-position.

4.2.6. Dough fermentation characteristics

Gaseous release and dough development of dough formulations were measured using a rheofermentometer F3 (Chopin, Villeneuve-La-Garenne Cedex, France). Dough samples were prepared as described previously (section *Dough preparation*) with 300 g of flour and addition of dry yeast (1g/100 g flour mixture). After kneading, 315.0 ± 0.5 g of dough sample were placed in the fermentation basket and covered with the optical sensor. The proofing chamber was closed hermetically to begin the measuring series for 180 min at 30 ºC. Recorded values included: H_m = maximum dough height; H = dough height at the end of measurement; H'_m = maximum height of gaseous release; T_1 = time to reach H_m; T'_1 = time of H'_m; T_x = time of gas release. Measurements were performed in triplicate.

4.2.7. Bread production

Dough samples for bread production were prepared as previously described (section *Dough preparation*) with 400 g of flour and addition of dry yeast (1g/100 g flour mixture). After kneading, 200 g of dough was weigh into a baking tin (height/width/depth 11.0 cm/7.0 cm/8.0 cm) and proofed for 45 min at 30 °C with 80% relative humidity. Baking was performed for 26 min at 230 ºC with 0.5 l initial steam in a deck Matador 12.8 oven (Werner & Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany). Bread samples were cooled on a wooden rack for 1 h at room temperature before bread volume, crumb texture, and crumb structure image analyses were performed. Baking trials were conducted in triplicate.

4.2.8. Bread characteristics

Bread volume was measured with a laser-based volumeter (BVM-L 370, Perten Instruments, Hägersten, Sweeden). Specific volume was then calculated as division of volume by the weight of each bread sample. Crumb texture profile analysis was assessed using a TVT-300 XP texture analyser (Perten Instruments, Hägersten, Sweeden) equipped with a 20 mm aluminium cylindrical probe. Two 12.5 mm bread slices were compressed by 40 % in two subsequent cycles with 15-s intermediate rest. Two replicates from three breads of each type were analysed. To study the crumb structure, images were captured in the RGB (24 bit) standard format using a flatbed scanner (Canon Scan N67OU, Netherlands) with a resolution of 600 dpi and supporting software (Canon Scan Toolbox version 4.1). For each image, a single 1000x1000 pixel field of view was cropped from the image centre and converted to a 256 level grey scale. Image segmentation of each field of view was performed with Otsu’s method using Matlab (207). Afterwards, cell morphological parameters were obtained with image processing toolbox 9.4 from Matlab. Three replicates from three breads of each type were analysed.

4.2.9. Statistical analysis

All dependent variables from every dough and bread value analysed were tested for distribution of the residuals with Shapiro–Wilk's test. Replacement levels of wheat flour by the different extracts were studied using a one-way analysis of variance (ANOVA), if normal distribution of the residuals was confirmed. Welch correction was applied when homogeneity of variances was not verified. Whenever statistical significances were found, Tukey´s or Dunnett T3 post-hoc tests were applied for mean comparison, depending on equal variances assumption or not.

If normal distribution of the residuals was not found, replacement levels of wheat flour by the different extracts were studied using a Kruskal–Wallis test. Whenever statistical significances were found, Mann–Whitney's test post-hoc test was applied for median comparison.

PLS regression was also used to study the relationships between bread parameters (Y-matrix) and bread dough parameters (X-matrix) in terms of prediction of Y-variables from X-variables. Random validation was also applied to identify relevant X-variables. Scores and loading plots were analysed, as well as, calibration and validation coefficients.

ANOVA and Kruskal–Wallis analyses were performed at 5% significance level, using SPSS for Windows, version 22 (IBM Corporation, New York, USA). PLS regression analyses were conducted with the XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France) at 5% significance level.

4.3. Results and discussion

4.3.1. Extract composition

Results from dietary fibre (DF) fractions, sodium, and chlorine are presented in Table 4.1. Regarding total dietary fibre (TDF), the content in orange extract (OE), yeast extract (YE), and pomegranate extract (PE) was above the 50 g/100 g of food product level required to be regarded as a rich source of fibre (233). The IDF fraction ranged between 66.48 and 98.41% of TDF. Elderberry extract (EE) values for DF were lower than reported (234). For OE, the content of TDF, insoluble dietary fibre (IDF), and soluble dietary fibre (SDF) were in the same range of what is reported in literature (235, 236). Although TDF was in accordance with values described in literature, SDF values obtained for PE and YE were lower (- 86% and -73%, respectively) (237-240). The use of water in the extraction procedures could explain this values.

(1-3)-β-D-Glucans are a major structural component in cell walls and are ubiquitous in nature, particularly in algae, fungi, yeast, and cereals. (1-3)(1-4)-β-D-Glucan (1,3:1,4BG) occurs in cereals, while (1-3)(1-6)-β-D-Glucan ((1,3:1,6BG) is mainly present in yeast and fungi (241). As expected, since none of the extracts were obtained from cereals, values found for 1,3:1,4BG were low (below 0.1 g/100 g extract). Although 1,3:1,4-BG impairs bread quality, due to its properties comparable to hydrocolloids (242), the low 1,3:1,4-BG content would probably not have an impact on dough and bread characteristics. Concerning (1-3)(1-6)-β-D-Glucan and (1-3)-β-D-Glucans (1,3:1,6BG) content in EE, OE, and PE was also low (below 7 g/100 g extract). Higher values were found in YE (median of 37.98 g/100g extract), which were in accordance with values reported by Thammakiti *et al.* (204).

With respect to sodium content, OE and PE were in same range of values reported by Chiocchetti *et al.* (243), and Hasnaoui *et al.* (238), respectively. In EE and YE, sodium was lower than reported by Sun-Waterhouse *et al.* (22), and Amorim *et al.* (244), respectively. Regarding chlorine, no values were found in literature for these extracts. Sodium chloride

(NaCl) is known to affect gluten behaviour, since it strongly affects gluten network development during mixing, thus influencing the whole bread production (245, 246). Furthermore, it has a strong impact on bread flavour (247). Considering the flour replacement, sodium chloride addition to the system was lower than 0.01 g/100 g flour mixture. Thus, no technological or sensorial influence is expected.

Table 4.1. Dietary fibre fractions, chlorine and sodium content (g/100 g), on dry weight basis.

Extract	TDF	IDF	SDF	(1,3:1,4BG)[1]	(1,3:1,6BG)[1]	Na	Cl
Elderberry (EE)	40.94 ± 0.89^{a}	29.29 ± 0.31^{a}	11.65 ± 0.59^{a}	0.009^{a} (0.008-0.010)	0.88^{a} (0.88-1.00)	0.470 ± 0.001^{a}	0.024 ± 0.015^{a}
Orange (OE)	72.92 ± 0.38^{b}	70.78 ± 0.61^{b}	2.14 ± 0.42^{b}	0.018^{b} (0.016-0.020)	5.47^{b} (5.21-5.57)	0.084 ± 0.002^{b}	0.009 ± 0.016^{b}
Pomegranate (PE)	59.03 ± 1.47^{c}	51.67 ± 1.10^{c}	7.37 ± 2.31^{ab}	0.007^{a} (0.007-0.008)	6.14^{b} (4.18-6.27)	0.657 ± 0.004^{c}	0.344 ± 0.132^{c}
Yeast (YE)	72.33 ± 2.51^{b}	52.25 ± 2.50^{c}	20.09 ± 4.98^{ab}	0.000^{c} (0.000-0.000)	37.98^{c} (37.73-39.02)	1.109 ± 0.006^{d}	0.002 ± 0.012^{b}
p	<0.001**	<0.001**	<0.001**	0.016***	0.023***	<0.001*	<0.001**

Data expressed as mean ± standard deviation, n = 3.

[1] data expressed as median (minimum-maximum).

1,3:1,6BG: (1-3)(1-6)-β-D-Glucan and (1-3)-β-D-Glucans; 1,3:1,4BG: (1-3)(1-4)-β-D-Glucan; BP, By-product; IDF, Insoluble dietary fibre; SDF, Soluble dietary fibre; TDF, Total dietary fibre.

Different letters for each extract in a column show statistically significant differences ($p < 0.05$) between means in normal distribution or medians in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Dunnett T3's test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Mann-Whitney's test.

4.3.2. Dough behaviour

An increase in optimum water absorption (OWA), measured in DoughLAB, was observed (Table 4.2) as OE, PE, and YE replacement levels were increased. Compared to the control, the OWA increase was up to 27% for OE, 10% for YE, and 7% for PE. Addition of DF usually results in increased optimum water absorption, as they have high water-binding capacity due to the hydroxyl groups present, allowing more water interactions through hydrogen bonding (111). For EE, an opposite tendency was found in OWA that decreased 31% with increasing DF content. This could be related to DF chemical structure, association between molecules, particles size, and DF porosity (248). According to Tańska *et al.* (234), lignin is the main DF fraction in elderberry juice pomace is (nearly 24.4 g/100 g dry matter) and cellulose (22.5 g/100 g dry matter). These values are higher than those reported by Frølich *et al.* (249) for whole grain: 0.8 g/100 g dry matter for lignin and 2.5 g/100 g dry matter for cellulose. Lignin is water insoluble and has hydrophobic binding capacity (113). Therefore, with an increase in this fraction, a decrease in OWA would be expected.

Gluten is the skeleton of wheat dough, giving it an elastic structure. Replacement of wheat flour with fibre rich ingredients affects gluten network formation by gluten dilution, competition for water and or mechanical disruption of the gluten network (250, 251). Hence, EE, PE, and YE incorporation might decrease dough cohesiveness and swelling capacity, resulting in a shortened development time (Table 4.2). For OE, there was an increase of development time. This could be due higher water uptake of DF present in OE. Rosell *et al.* (111) had reported a delay in gluten network development caused by the competition for water between DF and protein.

Table 4.2. Effect of different replacement levels of wheat flour by extract (% of wheat flour) on optimum water absorption and dough development time, on an adjusted moisture basis

Extract (% wheat flour)	Optimum Water Absorption (%)	Development time (s)
Elderberry (EE)		
0[1]	59.0	214 ± 77
4	57.0	282 ± 21
36	41.0	104 ± 3
Orange (OE)		
0[1]	59.0	214 ± 77
4	67.0	636 ± 68
8	75.0	480 ± 51
Pomegranate (OE)		
0[1]	59.0	214 ± 77
4	61.0	128 ± 7
16	63.1	122 ± 7
Yeast (YE)		
0[1]	59.0	214 ± 77
4	65.0	124 ± 7

Data expressed as mean ± standard deviation, fresh weight, n = 3.

[1] Control dough.

4.3.3. Dynamic mechanical thermal analysis (DMTA) with oscillatory measurements

Fig. 4.2 shows the rheological results of temperature ramps for doughs prepared with different extracts and at different replacement levels of wheat flour by extract. Complex shear modulus ($|G^*|$) represents the dough firmness and is the result of the vectorial sum of storage modulus (G') and loss modulus (G''). Overall, during initial heating (up to 57 °C) $|G^*|$ decreased slightly with temperature increase. This decrease continued until the onset of starch gelatinization, where a significant increase of $|G^*|$ ($|G^*|_{onset}$) was observed. Maximum starch gelatinization was observed when maximal structural firmness and strength was reached, represented by maximum $|G^*|$ value ($|G^*|_{max}$). Loss factor tan δ (tan δ) is another important parameter to consider as it is related with the overall visco-elastic response. Regardless of extract and replacement level, tan δ was always below 1, indicating a solid, elastic-like behaviour from doughs. In general, tan δ values were almost constant with just slight variations until reaching the highest viscous ratio and lowest structural stability, represented by the maximum of loss factor tan δ (tan δ_{max}).

$|G^*|_{onset}$ mean values were higher than control (13.3×10^3 Pa) for every extract and replacement level. Significant differences ($p < 0.05$) were found for 16% PE and 4% EE (80.0×10^3 Pa and 15.0×10^3 Pa, respectively). For $|G^*|_{max}$, mean values were also higher than control (114.3×10^3 Pa), except for EE. Differences were significant ($p < 0.05$) for 8% OE, 4% EE, and 36% EE (136.3×10^3 Pa, 95.3×10^3 Pa, and 96.8×10^3 Pa, respectively). This could mean that the effect of starch gelatinization was higher at $|G^*|_{max}$. In dough containing OE, PE, and YE, the water uptake was higher (as seen by the OWA, Table 4.1), and thus, there could be enough water in the system for an increased starch gelatinization and dough strength (higher $|G^*|_{max}$). An opposite behaviour was observed for EE, where a lower water uptake (Table 4.2) could result in less water available in the system and decreased starch gelatinization. Average temperature values at $|G^*|_{onset}$ and $|G^*|_{max}$ were not statistically different ($p > 0.05$) from control (54.58 ºC and 87.56 ºC). Results obtained are in agreement to Ahmed *et al.* (252) findings, where water insoluble date fibre incorporation significantly increased dough mechanical strength during heating. It was concluded that fibre incorporation has no effect on gelatinization temperature, but reinforced blended dough mechanical strength. However, this explanation may not explain the lower $|G^*|_{max}$ values found for EE.

Comparing median tan δ_{max} values with control (0.409), a significant ($p < 0.05$) decrease was detected for every extract and replacement level, except for 4% EE (0.442) and 4% OE (0.412). Regarding temperature, an early arrival to tan δ_{max} was also registered for every extract and replacement level, except for 36% EE ($p > 0.05$). A decrease in tan δ_{max} and temperature was also reported by Migliori and Gabriele (253) when adding pentosane powder. A smooth shape after tan δ_{max} revealed a slower transition of the dough to a solid-like behaviour.

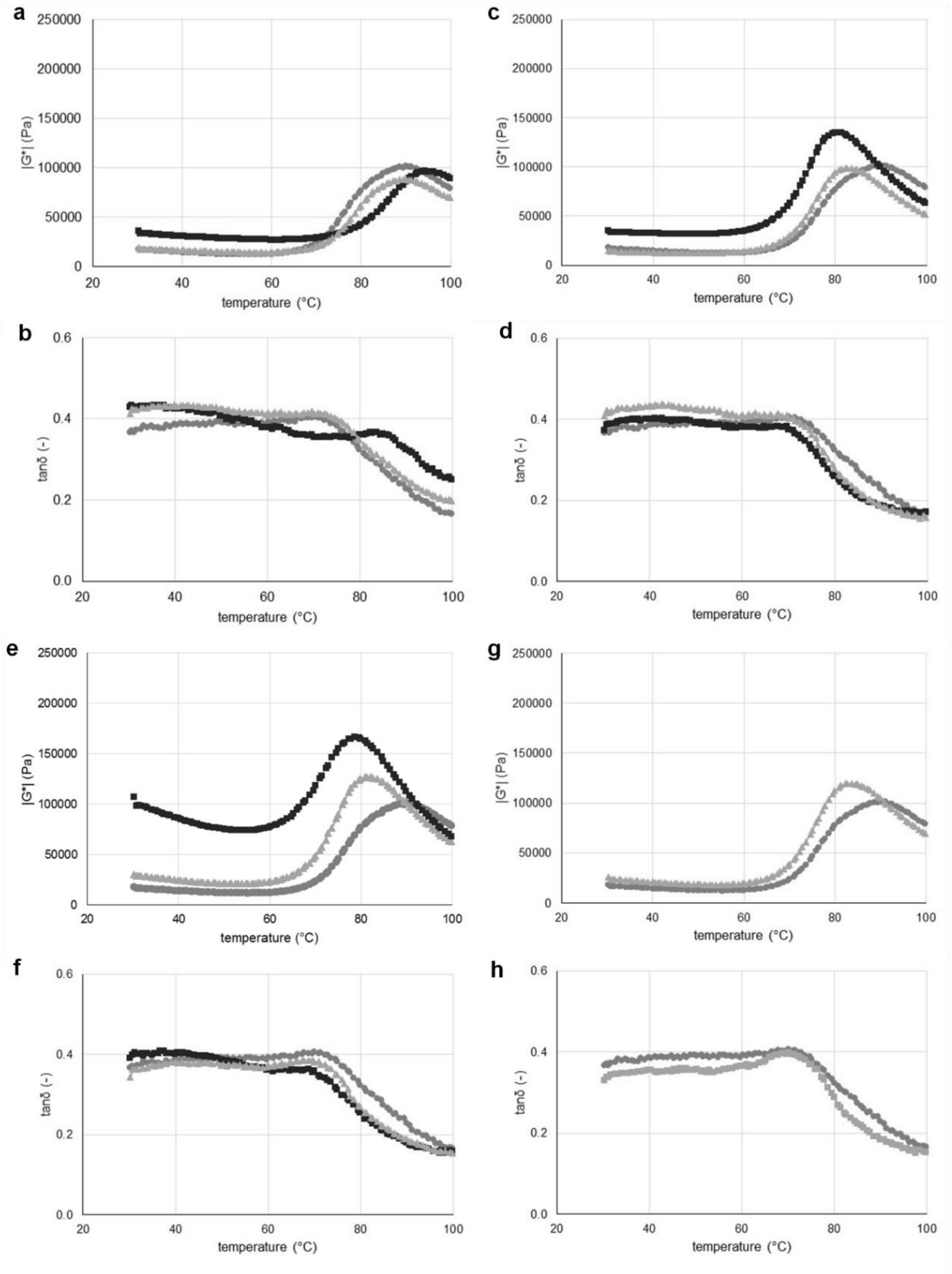

Figure 4.2. Complex shear modulus |G*| (Pa) (**a**, **c**, **e**, and **g**) and loss factor tanδ (**b**, **d**, **f**, and **h**) of dough prepared with different extracts and at different replacement levels of wheat flour by extract (% of wheat flour), as a function of the temperature during a heating step (4.25 ºC/min). Control dough values were represented by ●. **a** and **b**) Elderberry extract (EE) at: ▲ 4 %, ■ 36 %; **c** and **d**) Orange extract (OE) at: ▲ 4 %, ■ 8 %; **e** and **f**) Pomegranate extract (PE) at: ▲ 4 %, ■ 16 %; **g** and **h**) Yeast extract (YE) at: ▲ 4 %. Data expressed as mean ± standard deviation, on dry weight basis, n = 3

4.3.4. Protein microstructure analysis

Visual analysis of dough microstructure using confocal laser scanning microscopy (CLSM) provided information and allowed visualization of the developed protein network in each extract and replacement level. CLSM analysis indicated structural changes to varying extents, depending on extract and replacement level, and were in accordance with results from fundamental rheology (values obtained from initial DMTA measurements, as shown Table 4.3). In micrographs (Figure 4.3), protein network appears in grey and white. Black holes in the continuous network indicate embedded starch granules, whereas the white rings around a black hole signals entrapped air bubble. In the control (micrograph a), the protein network was well formed and some empty areas between protein strands could be detected. With 4% YE (micrograph b), protein network was more spread than control. Even though the water addition to dough was adjusted for a specific torque during kneading, the dough firmness in a rheometer showed a 43% higher $|G^*|$ at 30 ºC ($|G^*|_{30\,ºC}$) compared to the standard dough ($p < 0.005$). This could be related with a higher water absorption of the polymers over time. These differences could also lead to an intensified mechanical energy input in the system and thus a more spread protein network, as seen in micrograph 2. For doughs with OE (micrographs c and d), protein network had some differences compared to the control. In dough containing 4% OE (micrograph d), protein network appeared more spread and distributed, but a bit weakened ($|G^*|_{30\,ºC}$ lower and tan δ higher than control, with $p > 0.005$). With an increase in OE to 8% (micrograph c), protein network was more clustered and stretched ($|G^*|_{30\,ºC}$ 96% higher than control, with $p < 0.005$). For doughs with PE (micrographs e and f), proteins exhibited strong differences compared to the control. For 4% PE (micrograph f), protein network started to get inhibited, with proteins with some irregularity in distribution, and partially agglomerated. Still, proteins appeared to be more stretched ($|G^*|_{30\,ºC}$ higher and tan δ lower than control, with $p > 0.005$). For 16% PE, no protein network was visible. Anthocyanins present in elderberry are fluorescent with $\lambda_{emission}$ in the same range as the one used for protein microstructure analysis. So, the protein structure of dough formulations with EE was, at some extent, masked by the anthocyanins (white granules in micrographs g and h). Protein network in dough with 4% EE appeared less interconnected (tan δ 13% higher than control, with $p < 0.005$). The same tendency was observed for 36% EE (tan δ 17% higher than control, with $p < 0.005$), which also showed an inhibition in protein network formation along with protein agglomeration. The overall increase in dough firmness, visible in microstructure analysis, may limit dough free expansion during fermentation and impair doughs baking performance (116).

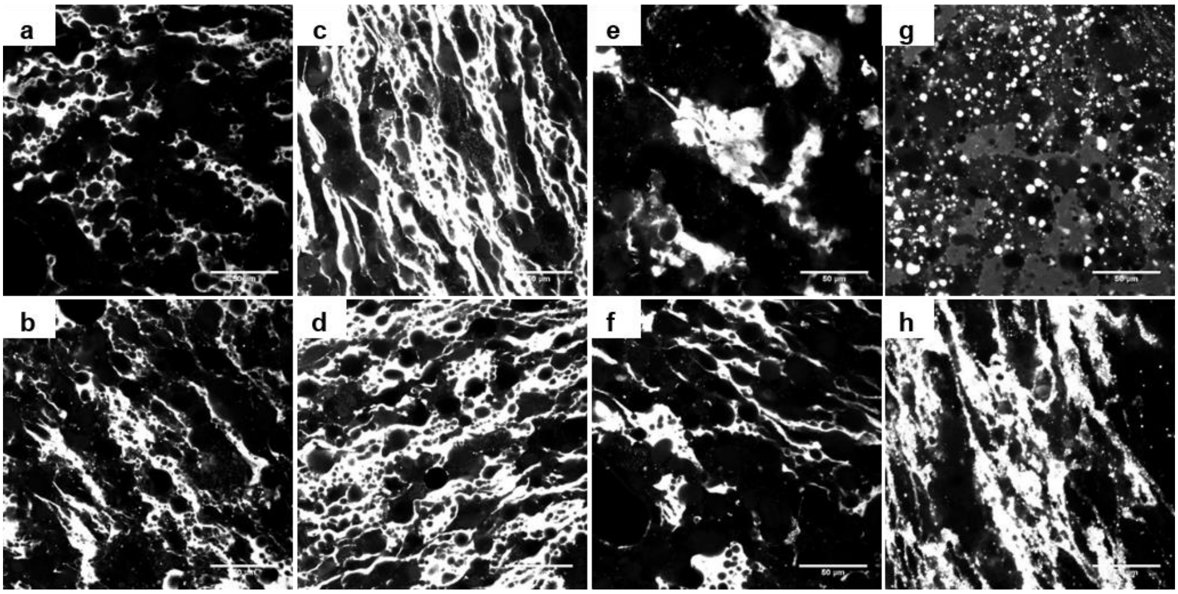

Figure 4.3. Grey scale CLSM micrographs of control dough and dough with different replacement levels of wheat flour by extract (% of wheat flour), on dry weight basis. Each micrograph has a size of 215 µm x 215 µm. The protein network (stained with rhodamine B or nile blue) is shown in grey and white; black holes in the continuous network indicate embedded starch granules, whereas the white rings around a black hole signals entrapped air bubble. **a**) Control; **b**) Yeast extract (YE) at 4%; **c**) Orange extract (OE) at 8%; **d**) OE at 4%; **e**) Pomegranate extract (PE) at 16%; **f**) PE at 4%; **g**) Elderberry extract (EE) at 36%; **h**) EE at 4%

Table 4.3. Values for complex shear modulus $|G^*|$ and loss factor tanδ measured at 30 ºC of control dough and dough with different replacement levels of wheat flour by extract (% of wheat flour), on dry weight basis

Extract (% wheat flour)	**$\|G^*\|_{30\ ^{\circ}C}$ (x10^3 Pa)**	**$\tan\delta_{30\ ^{\circ}C}$**
Elderberry (EE)		
0^1	17.0 (15.0−21.6)	0.366 ± 0.007^a
4	13.3 (11.9−31.5)	0.413 ± 0.018^b
36	31.5 (31.4−44.8)	0.429 ± 0.019^b
p	ns	0.007^*
Orange (OE)		
0^1	17.9 ± 3.3^a	0.366 ± 0.007
4	14.5 ± 1.7^a	0.407 ± 0.018
8	35.1 ± 9.9^b	0.372 ± 0.024
p	0.013^*	ns
Pomegranate (PE)		
0^1	17.9 ± 3.3^a	0.366 ± 0.007
4	30.0 ± 2.8^b	0.342 ± 0.033
16	107.6 ± 26.1^c	0.390
p	0.010^*	ns
Yeast (YE)		
0^1	17.9 ± 3.3^a	0.366 ± 0.007^a
4	25.7 ± 3.3^b	0.328 ± 0.005^b
p	0.034^*	0.002^*

Data expressed as mean ± standard deviation, (n=3).

[1] Control dough.

$|G^*|_{30\ ^{\circ}C}$, complex shear modulus measured at 30 ºC; ns, not significant; **$\tan\delta_{30\ ^{\circ}C}$**, loss factor tanδ measured at 30 ºC.

Different letters for each extract in a column show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Dunnett T3's test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

4.3.5. Dough fermentation characteristics

The influence of each extract and replacement level on the fermentation characteristics was recorded by the rheofermentometer and is shown in Table 4.4. Compared to the control, maximum dough height (H_m) significantly decreased ($p < 0.005$) in in every case (36% EE: -49%; 4% OE: -23%; 8% OE: -46%; 4% PE: -34%; 16% PE: -90%; 4% YE: -34%), except for 4% EE. Incorporation of fractions rich in DF may dilute gluten network and/or promote interactions between DF components and gluten (19, 254). This dilution and/or interaction effect limit dough free expansion throughout fermentation, showing a decrease in H_m, which is a marker for baking quality (116).

The same decrease tendency was observed for final dough height (h), except for a 12% increase ($p < 0.005$) in 4% EE. Apart from 4% EE, these results were in agreement with the rheological behaviour measured and may be due to DF content present in extracts (116, 255). For 4% EE, the tan δ increase revealed a weakening in dough structure that could influence dough development throughout fermentation. Here, DF profile could be more relevant than concentration used, as reported by Sidhu and Bawa (256). These authors observed a significant increase in H_m and h for wheat dough with xanthan gum incorporation, up to 0.5%.

The time to reach maximum dough development (T_1) significantly increased ($p < 0.005$), compared to the control, for 36% EE (52%), 4% PE (49%), 16% PE (52%), and 4% YE (51%). The opposite was observed for 4% OE, registering a 16% decrease ($p < 0.005$).

Regarding gas behaviour, maximum height of gaseous production (H'_m) was significantly lower ($p < 0.005$) for 36% EE (-51%) and 4% PE (-58%), and did not modify the remaining. This decrease was in accordance with results reported by Föste *et al.* (257) for quinoa bran dough. The time of maximum gas formation (T'_1) significantly increased ($p < 0.005$) with 36% EE (83%) and 4% PE (33%), which had also H'_m decreased. In relation to the time at which gas starts to escape from dough (T_x), it was not affected by any extract or replacement level. Data from gas behaviour might indicate lower substrate availability for yeast growth in 36% EE and 4% PE, taking more time to reach T'_1. Statistical treatment of the results indicate that globally, wheat flour replacement had more impact on dough development than on gas production throughout fermentation. This reflected what was observed in rheology and microstructure analyses.

Table 4.4. Values for dough development and gas production throughout fermentation of control dough and dough with different replacement levels of wheat flour by extract (% of wheat flour), on dry weight basis

Extract (% wheat flour)	**H_m (mm)**	**h (mm)**	**T_1 (min)**	**H'_m (mm)**	**T'_1 (min)**	**T_x (min)**
Elderberry (EE)						
0^1	61.1 ± 1.8^a	46.8^a (40.0−48.2)	119.4^a (114.0−160.8)	76.5 ± 3.5^a	84.6 ± 5.1^a	76.5 ± 4.5
4	63.8 ± 1.0^a	52.9^b (49.2−53.4)	109.8^a (108.0−118.8)	31.5 ± 23.6^{ab}	104.2 ± 10.1^a	89.2 ± 12.4
36	31.4 ± 1.0^b	31.0^a (30.7−32.6)	180.0^b (180.0−180.0)	37.3 ± 3.8^b	155.2 ± 22.0^b	117.0 ± 0.0
p	$<0.001^*$	0.043^{***}	0.027^{***}	0.001^{**}	0.001^*	ns
Orange (OE)						
0^1	61.1 ± 1.8^a	47.3 ± 0.9	119.4^a (114.0−160.8)	76.5 ± 3.5	84.6 ± 5.1	77.3 (72.0−81.0)
4	47.2 ± 1.6^b	39.7 ± 2.0	99.0^b (96.0−106.8)	39.8 ± 32.7	91.6 ± 35.1	63.0
8	33.3 ± 3.2^c	29.5 ± 1.2	129.0_a (121.8−133.8)	73.6 ± 1.3	74.2 ± 3.1	61.8 (60.0−61.8)
p	$<0.001^*$	ns	0.043^{***}	ns	ns	ns
Pomegranate (PE)						
0^1	61.4^a (59.2−62.7)	46.8 (40.0−48.2)	119.4^a (114.0−160.8)	76.5 ± 3.5^a	84.6 ± 5.1^a	77.3 (72.0−81.0)
4	40.5^b (39.2−41.2)	40.3 (39.2−41.1)	177.0^b (168.0−180.0)	32.3 ± 12.8^b	114.0 ± 18.0^b	80.1 (75.0−85.2)
16	6.4^c (6.4−7.1)	6.4 (6.4−7.1)	180.0^b (180.0−180.0)	70.0 ± 0.9^{ab}	96.0 ± 6.0^{ab}	48.0 (48.0−109.5)
p	0.018^{***}	ns	0.022^{***}	0.017^{**}	0.013^*	ns
Yeast (YE)						
0^1	61.1 ± 1.8^a	46.8 (40.0−48.2)	119.4^a (114.0−160.8)	76.5 ± 3.5	84.6 ± 5.1	76.5 ± 4.5
4	40.6 ± 0.5^b	40.1 (40.0−40.1)	178.8^b (175.5−178.8)	33.2 ± 21.3	135.6 ± 34.9	81.9 ± 1.3
p	$<0.001^*$	ns	0.032^{***}	ns	ns	ns

Data expressed as mean ± standard deviation or as median (minimum−maximum), n = 3.

[1] Control dough.

H_m, maximum dough height (mm); h, final height of dough (mm); H'_m, maximum height of gaseous production (mm); ns, not significant; T_1, time to reach the maximum dough height (min); T'_1, time to reach the maximum gas formation rate (min); T_x, time of porosity (gas starts to escape the dough matrix) (min).

Different letters for each extract in a column show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey’s test, since homogeneity of variances was confirmed by Levene’s test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Dunnett T3’s test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Mann-Whitney’s test.

4.3.6. Bread characteristics

One of the main problems of DF addition in breadmaking is the significant reduction of bread volume and specific volume. Pomeranz *et al.* (122) suggested that this reduction in volume and specific volume occurs by dilution of the gluten content and change in crumb structure, which in turn impairs carbon dioxide retention. Also, Chen *et al.* (123) suggested that TDF molecules may interact with wheat flour proteins and interfere with gluten development. As seen in Table 4.5, there was a significant decrease ($p < 0.005$) in bread volume and specific volume, compared to the control, namely for 36% EE (-16%; -17%), 8% OE (-9%; -10%), 4% PE (-6%; -9%), 16% PE (-36%; -38%). Compared to the control, bread volume for 4% EE significantly increased, which was in agreement with dough H_m and h. Bread volume increase with increase of H_m and h was also reported by Sidhu and Bawa (256). Bread volume for 4% OE and 4% YE also increased significantly compared to the control, although no significant differences were observed for h.

Crumb image analysis was performed on each scanned slide, in order to have a detailed view of bread structure (Figure 4.4). Extract replacement altered breads colour and height characteristics. Crumb colour was darker for every extract and replacement level, compared to the control. Particularly, EE breads (samples g and h, from Figure 4.4) exhibited an unusual bread colour, which could be interesting to the formulation of specialty products. Observations regarding height are in agreement with bread volume. Here, 4% YE and 4% OE were close to the control, while 4% EE was higher. A decrease in bread height was observed for PE, 36% EE, and 8% OE. Bread cell attributes (Table 4.5), especially cell size should have a significant impact in bread texture and mouthfeel perception (209). The total number of cells did not present major changes from the control, apart from 4% YE (-31%) and 36% EE (21%). Considering cell area distribution, 84.60% to 98.75% of the cells in the different extract bread formulations, and 97.08% in control had an area ≤ 5.0 mm^2 (considered as small cells). Significant increase ($p < 0.005$) in cells with area > 5.0 mm^2 (large cells) was detected for 36% EE, 4% OE, 16% OE, and 4% YE, implying that larger gas cells occurs during baking (209). Bread cells features depend on gluten network gas retention capacity, as well as on maintenance of gas cells integrity throughout fermentation and baking. If gas cell integrity is not conserved, small gas cells will coalesce into larger ones. The greater the gas cells coalescence is, the coarser is the bread crumb structure. So, lower dough H_m and h results found for 36% EE, 4% OE, 16% OE, and 4% YE might explain the higher percentage on large cells.

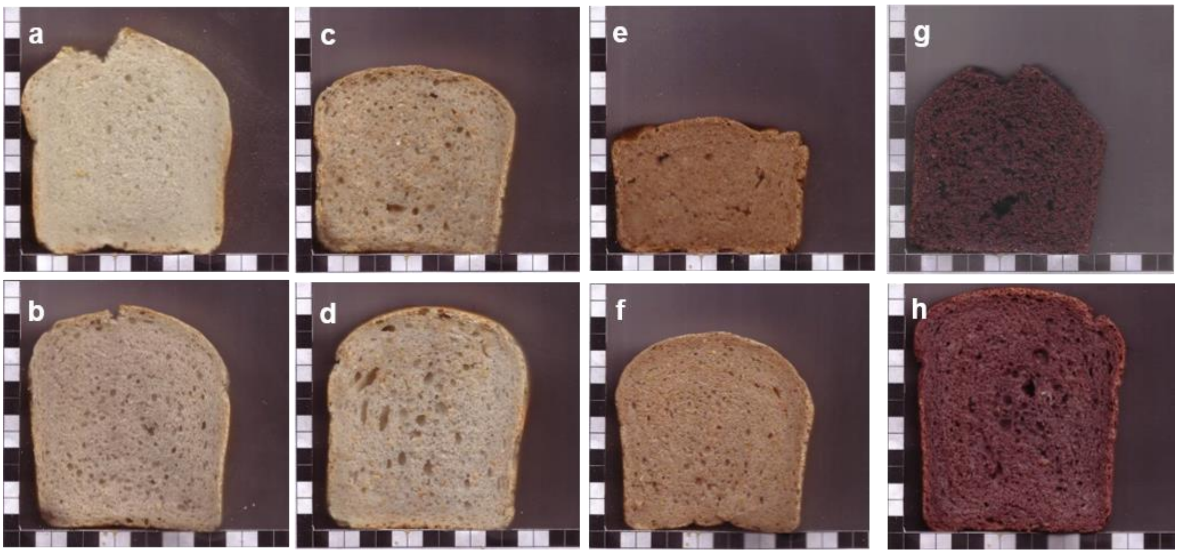

Figure 4.4. Bread slices scanned images. a) Control; b) Yeast extract (YE) at 4 %; c) Orange extract (OE) at 8 %; d) OE at 4 %; e) Pomegranate extract (PE) at 16 %; f) PE at 4 %; g) Elderberry extract (EE) at 36 %; h) EE at 4 %. Images were taken 1.5 h after baking

4.3.7. Bread texture

The bread crumb texture profile was also studied (Table 4.5). The crumb hardness was significantly higher (*p* < 0.005) than control for 8% OE (29%), 4% PE (36%), and 16% PE (223%) and lower for 4% EE (-40%). The registered increase in hardness may be due to the addition of DF with higher water absorption. Dilution of the gluten content and crumb structure disruption may occur, with a thickening of the walls surrounding gas cells that impair gas retention (111). Cohesiveness only changed significantly (*p* < 0.005) from control for 36% EE (-30%), 4% PE (-7%), 8% PE (-30%), whereas chewiness values for 4% EE (-39%) and 36% (-50%) were lower than control, while 8% OE (35%) and 16% PE (124%) was higher.

Table 4.5. Values for bread physical parameters, crumb texture, and crumb image analysis of control dough and dough with different replacement levels of wheat flour by extract (% of wheat flour), on dry weight basis

Extract (% wheat flour)	**Volume (mL)**	**Specific Volume (mL/g)**	**Number of cells**	**Cell area (% of total cells)**					**Hardness (N)**	**Cohesiveness**	**Chewiness (J)**
				CA ≤ 0.1 (mm²)	**0.1 < CA ≤ 1.0 (mm²)**	**1.0 < CA ≤ 3.0 (mm²)**	**3 .0< CA ≤ 5.0 (mm²)**	**CA > 5.0 (mm²)**			
Elderberry (EE)											
0[1]	329.77 ± 1.81^{a}	1.87 ± 0.10^{a}	495^{a} (281−686)	76.70 ± 5.03	19.82 (10.37−20.77)	3.23 ± 0.61^{a}	0.71^{a} (0.30−1.41)	2.92 ± 1.01^{a}	11.11 ± 0.73^{a}	0.789 ± 0.004^{a}	7.44 ± 0.48^{a}
4	405.65 ± 11.21^{b}	2.22 ± 0.07^{a}	270^{a} (220−366)	79.82 ± 1.72	16.91 (14.90−19.08)	1.94 ± 0.57^{b}	0.15^{b} (0.00−0.46)	1.26 ± 0.39^{b}	6.62 ± 0.39^{b}	0.786 ± 0.011^{a}	4.57 ± 0.30^{b}
36	276.94 ± 4.65^{c}	1.56 ± 0.02^{b}	621^{b} (433−685)	74.16 ± 7.18	15.67 (8.73−25.68)	3.29 ± 1.13^{a}	0.74^{a} (0.00−2.24)	5.27 ± 1.74^{c}	12.21 ± 0.97^{a}	0.549 ± 0.015^{b}	3.71 ± 0.26^{c}
p	<0.001*	<0.001**	0.001 ***	ns	ns	0.002*	0.008***	<0.001**	<0.001*	<0.001**	<0.001*
Orange (OE)											
0[1]	329.77 ± 1.81^{a}	1.87 ± 0.10^{a}	475 ± 169	76.70 ± 5.03^{a}	19.82 (10.37−20.77)	3.23 ± 0.61	0.68 ± 0.37	2.92 ± 1.01^{a}	11.11 ± 0.73^{a}	0.789 ± 0.004	7.36^{a} (6.74−8.18)
4	375.24 ± 12.94^{b}	2.07 ± 0.06^{b}	330 ± 54	66.88 ± 3.99^{b}	22.68 (12.17−29.10)	3.95 ± 1.50	1.03 ± 0.44	4.97 ± 2.44^{b}	9.39 ± 2.70^{a}	0.784 ± 0.031	6.46^{a} (4.79−7.95)
8	299.01 ± 4.77^{c}	1.68 ± 0.36^{c}	322 ± 79	64.24 ± 66.88^{b}	26.97 (21.84−29.17)	4.07 ± 1.50	1.04 ± 0.57	4.34 ± 1.83^{ab}	15.47 ± 1.05^{b}	0.766 ± 0.017	10.21^{b} (9.03−10.99)
p	<0.001*	<0.001*	ns	<0.001*	ns	ns	ns	0.009*	<0.001*	ns	0.003***

Table 4.5. Values for bread physical parameters, crumb texture, and crumb image analysis of control dough and dough with different replacement levels of wheat flour by extract (% of wheat flour), on dry weight basis (continued)

Extract (% wheat flour)	Volume (mL)	Specific Volume (mL/g)	Number of cells	Cell area (% of total cells)					Hardness (N)	Cohesiveness	Chewiness (J)
				CA ≤ 0.1 (mm^2)	0.1 < CA ≤ 1.0 (mm^2)	1.0 < CA ≤ 3.0 (mm^2)	3 .0< CA ≤ 5.0 (mm^2)	CA > 5.0 (mm^2)			
Pomegranate (PE)											
0^1	329.77 ± 1.81^a	1.87^a (1.86−1.88)	495 (281−686)	73.33^a (69.72−84.52)	19.82 (10.37−20.77)	3.23 ± 0.61^a	0.68 ± 0.37	2.92 ± 1.01^a	11.11 ± 0.73^a	0.789 ± 0.004^a	7.44 ± 0.48^a
4	309.35 ± 0.84^b	1.70^b (1.69−1.73)	416 (352−599)	77.19^a (70.52−88.00)	18.56 (10.90−24.37)	1.78 ± 0.74^b	0.66 ± 0.44	1.48 ± 1.08^b	14.04 ± 1.16^b	0.734 ± 0.025^b	8.34 ± 0.34^a
16	211.08 ± 4.77^c	1.17^c (1.12−1.17)	451 (398−528)	65.09^b (60.11−93.50)	25.46 (4.60−29.97)	4.25 ± 1.77^a	0.90 ± 0.53	3.41 ± 1.03^a	35.84 ± 5.23^c	0.550 ± 0.054^c	16.70 ± 1.03^b
p	<0.001*	0.027***	ns	0.009***	ns	0.001**	ns	0.002*	<0.001**	<0.001**	<0.001*
Yeast (YE)											
0^1	329.77 ± 1.81^a	1.87 ± 0.10	475 ± 169^a	76.70 ± 5.03^a	19.82^a (10.37−20.77)	3.23 ± 0.61^a	0.68 ± 0.37^a	2.92 ± 1.01^a	11.09 (9.97−12.22)	0.789 ± 0.004	7.44 ± 0.48
4	345.59 ± 4.90^b	1.89 ± 0.03	328 ± 48^b	63.95 ± 5.40^b	25.31^b (17.15−30.00)	4.39 ± 0.63^b	1.32 ± 0.45^b	5.58 ± 2.33^b	10.59 (10.39−10.70)	0.775± 0.030	0.13 ± 0.07
p	0.006*	ns	0.033**	<0.001*	0.002***	0.001*	0.005*	0.009**	ns	ns	ns

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=3, n=6, n = 9, respectively).

[1] Control bread.

CA, cell area; ns, not significant.

Different letters for each extract in a column show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Dunnett T3's test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Mann-Whitney's test.

4.3.8. Correlation of bread characteristics with dough parameters

Throughout this work, it was possible to observe the influence of EE, OE, PE, and YE on dough and bread, and their relationships. This influence could be related with TDF, IDF, and or SDF contribution of each extract. If so, the effect of DF on dough and bread would still be expected, independent of the extract used. Therefore, the influence of TDF, IDF, and SDF content on the relationships between dough and bread parameters were also considered and analysed, independent of the extract used. Multivariate PLS regression was performed in order to study the correlation of bread physical parameters, crumb texture, and crumb image analysis data with dough empirical and fundamental rheology, based on bread characteristics data prediction (Y-variables) from dough parameters data (X-variables). Contrary to linear regression, multivariate PLS regression is able to simultaneously evaluate the influence of more than one X-variable on Y-variables (258).

Table 4.6 summarises individual bread parameters prediction models from dough parameters. Of the 12 bread values analysed, all physical and texture, and 5 crumb image data were found to be correlated with dough measured parameters. Regression models were considered successful when $R^2 \geq 0.70$, and presenting good ability to predict new samples when $Q^2 \geq 0.50$ (259). In general, good regression models, with good predictive ability were found for volume, specific volume, hardness, cohesiveness, chewiness, and number of cells. Volume, specific volume, and hardness regression models performance was superior for TDF, presenting lower RMSE values (10.54 mL, 0.08 mL/g, and 2.93 N, respectively). For cohesiveness and chewiness, lower RMSE values for regression models were found with SDF (0.01 and 1.25 J, respectively). The only successful regression model, with good predictive ability for number of cells was detected with IDF with a RMSE value of 51.57. As discussed in previous sections, DF has an impact on bread volume and specific volume, and crumb hardness. Once TDF appeared to have a stronger influence than IDF or SDF, it could indicate that DF concentration as a total could be more relevant for these correlations than DF category (soluble or insoluble). Considering RMSE values, SDF appeared to have more impact in cohesiveness and chewiness, while number of cells was influenced by IDF.

The formulations used in bread dough substantially influence the crumb structure appearance (260). Cell distribution by area, in percentage, can provide useful information on dough properties. The presence of larger cells in bread crumb could evidence rupture of inter-cell dough films, resulting on gas cells coalescence on the final stages of proofing and during baking (260). However, correlation models for cell distribution by area, in percentage were poorly fitted ($R^2 < 0.50$), with poor ($0.00 < Q^2 < 0.50$) or lacking ($Q^2 < 0.00$) predictive ability. The only exception was observed for IDF in percentage of cells with area > 5 mm^2, where correlation model presented a good fit ($R^2 \geq 0.70$) but with poor predictive ability. These results could be due to a heterogeneity in cell distribution.

The importance of X-variables (dough parameters) in the projection and their correlation with Y-variables (bread parameters) was also determined, and latent variables were identified (Table 4.6). Moreover, dough parameters common in regression models with good performance for TDF, IDF, and SDF were identified. Correlations for every common dough parameter were analysed, in order to study their influence in bread parameters. So, doughs with lower resistance to deformation (lower $|G^*|_{30}$, $|G^*|_{onset}$, and $|G^*|_{max}$), higher viscous ratio (higher tan δ_{max}), higher maximum and final dough height (higher H_m and h), that required less time to reach maximum dough height (T_1), were the ones with higher volume and specific volume, and lower hardness (Table 4.6). Chewiness presented a correlation tendency similar to hardness for dough resistance to deformation, maximum and final dough height. Additionally, doughs with lower OWA, that reached the viscous ratio peak and maximum starch gelatinization at higher temperatures (higher Ttan δ_{max} and TG^*_{max}), produced breads with lower chewiness. Doughs that resulted in breads with higher cohesiveness were the ones with higher OWA, lower resistance to deformation (lower $|G^*|_{30}$ and $|G^*|_{onset}$), higher tan δ_{max}, H_m and h, and lower T_1. Dough resistance to deformation was not correlated with the number of cells, while doughs with lower DT, OWA, tan δ_{max}, higher Ttan δ_{max}, lower H_m and h, and higher T_1, resulted in bread with higher number of cells. Nevertheless, observed correlations do not necessarily imply causality. For that reason, correlation models obtained should be interpreted as showing associations rather than direct cause and effect relationship.

Table 4.6. Results of PLS regression between dough parameters (X-variables) and bread parameters (Y-variables) of bread formulations for the different dietary fibre fractions

Bread parameter	**Total dietary fibre**				**Insoluble dietary fibre**				**Soluble dietary fibre**			
	R^2	Q^2	RMSE	LV[1]	R^2	Q^2	RMSE	LV[1]	R^2	Q^2	RMSE	LV[1]
Physical parameters												
Volume	0.97	0.93	10.54	**h**(+); **tanδ**(+);**H_m**(+);**G^*_0**(-); **G^*_{onset}**(-); **T_1**(-); **G***(-); DT(+)	0.90	0.86	19.55	**h**(+); **H_m**(+); **tanδ**(+); **G^*_0**(-); **G^*_{onset}**(-); **T_1**(-); **G***(-); DT(+)	0.89	0.83	19.74	**H_m**(+); **tanδ**(+); **h**(+); **G^*_{onset}**(-); **G^*_0**(-); **G***(-); **T_1**(-)
Specific volume	0.93	0.90	0.08	**H_m**(+); **h**(+); **tanδ**(+);**G^*_0**(-); **G^*_{onset}**(-); **G***(-); **T_1**(-)	0.92	0.88	0.10	**H_m**(+); **tanδ**(+); **h**(+); **G^*_0**(-); **G^*_{onset}**(-); **G***(-); **T_1**(-)	0.87	0.81	0.10	**tanδ**(+); **h**(+); **H_m**(+);**G***(-); **G^*_{onset}**(-); **G^*_0**(-); **T_1**(-)
Texture parameters												
Hardness	0.85	0.79	2.93	**G^*_0**(+); **h**(-); **G^*_{onset}**(+); **H_m**(-); **G***(+); **tanδ**(-);	0.88	0.82	3.37	**h**(-); **G^*_{onset}**(+); **G^*_0**(+); **H_m**(-); **G***(+); **tanδ**(-); TG*(-)	0.85	0.76	3.38	**G^*_{onset}**(+); **h**(-); **G^*_0**(+); **H_m**(-); **G***(+); tanδ(-); TG*(-)
Cohesiveness	0.99	0.93	0.11	**tanδ**(+); **H_m**(+); **h**(+); **T_1**(-); **OWA**(+); **T'_1**(-); **G^*_0**(-); DT(+); G^*_{onset}(-); Ttanδ(-); tanδ_0(-);	0.96	0.84	0.02	**tanδ**(+);**T_1**(-); **OWA**(+); **T'_1**(-); **H_m**(+);**Ttanδ**(-); **h**(+); **G^*_0**(-); DT(+); G^*_{onset}(-)	0.99	0.94	0.01	**h**(+); **H_m**(+); **G^*_0**(-); **tanδ**(+); **G^*_{onset}**(-); **T_1**(-); tanδ_0(-); OWA(+);
Chewiness	0.84	0.51	1.25	**G***(+); **TG***(-); **OWA**(+); **G^*_0**(+); **h**(-); **Ttanδ**(-); **G^*_{onset}**(+);**H_m**(-); tanδ_0(-);	0.88	0.83	1.50	**G^*_0**(+); **G^*_{onset}**(+); **h**(-); **G***(+); **TG***(-); **H_m**(-); **Ttanδ**(-); H'_m(+); OWA(+);	0.88	0.81	1.25	**G***(+); **G^*_{onset}**(+); **G^*_0**(+); **h**(-); **H_m**(-); TG*(-); Ttanδ(-); OWA(+); tanδ(-)

Table 4.6. Results of PLS regression between dough parameters (X-variables) and bread parameters (Y-variables) of bread formulations for the different dietary fibre fractions

Bread parameter	**Total dietary fibre**				**Insoluble dietary fibre**				**Soluble dietary fibre**			
	R^2	Q^2	RMSE	LV[1]	R^2	Q^2	RMSE	LV[1]	R^2	Q^2	RMSE	LV[1]
Image analysis												
Number of cells	0.71	0.59	62.04	**Ttanδ**(+); **T_1**(+); **tanδ**(-); **OWA**(-); **H_m**(-); **DT**(-); T'_1(+); TG*(+); h(-)	0.79	0.68	51.57	**tanδ**(-); **Ttanδ**(+); **T_1**(+); **OWA**(-); **H_m**(-); **T'_1**(+); **h**(-); DT(-)	0.29	0.09	78.03	**H'_m**(+); **tanδ**(-); **G^*_0**(+); **OWA**(-); **G^*_{onset}**(+); **DT**(-); **G***(+); h(-); T_1(-);
CA ≤ 0.1	0.38	0.07	5.01	**OWA**(-); **DT**(+); **TG***(+); **Ttanδ**(+); **G***(-); **T'_1**(+); **H'_m**(+);	0.36	0.19	4.96	**H_m**(+); **h**(+); **OWA**(-); **G^*_{onset}**(-); **tanδ**(+); TG*(+); G*(-); G^*_0(-); T'_1(+)	0.44	0.31	5.26	**H_m**(+); **h**(+); **tanδ**(+); **G***(-); **OWA**(-); **G^*_{onset}**(-); **TG***(+); G^*_0(-)
1.0 < CA ≤ 3.0	0.24	0.02	0.86	**h**(-); **H_m**(-); **OWA**(+); **tanδ**(-); **DT**(+); **G^*_{onset}**(+); **H'_m**(+); G^*_0(+)	0.34	0.19	0.78	**H'_m**(+); **h**(-);**G***(+); **H_m**(-); **OWA**(+); **G^*_{onset}**(+);**TG***(-); T'_1(-)	0.42	0.28	0.90	**h**(-);**OWA**(+); **H'_m**(+); **H_m**(-); **G^*_{onset}**(+); **DT**(+); G^*_0(+); T'_1(-); tanδ(-); G*(+)
3.0 < CA ≤ 5.0	0.18	-0.53	0.35	**OWA**(+); **H_m**(-); **T'_1**(+); **H'_m**(-); **T_1**(+); **$\tan\delta_0$**(-); tanδ(-); TG*(-); h(-)	0.24	-0.60	0.34	**T'_1**(+); **H_m**(-); **H'_m**(-); **OWA**(+); **T_1**(+); **tanδ**(-);**h**(-)	0.20	-0.10	0.35	**$\tan\delta_0$**(+); **Ttanδ**(+); **T'_1**(+); **tanδ**(-); **H_m**(-); **h**(-)
CA > 5.0	0.21	-0.20	1.61	**tanδ**(-); **H_m**(-); **Ttanδ**(+); **h**(-); **T'_1**(+); T_1(+); DT(+)	0.71	0.17	1.00	**$\tan\delta_0$**(-); **DT**(+); **Ttanδ**(+);**H_m**(-);**h**(-); **OWA**(-); **tanδ**(-); **T'_1**(+);**G***(-)	0.26	-0.10	1.51	**tanδ**(-); **H_m**(-); **Ttanδ**(+); **h**(-); **T'_1**(+); T_1(+); DT(+)

Latent variables were only considered for good models; highly influential latent variables (Variable Importance for the Projection > 1) are represented in bold and the remaining are moderately influential latent variables (0.8 < Variable Importance for the Projection < 1); (+), positive correlation with Y-variable; (-), negative correlation with Y-variable.

DT, development time; G*, maximum |G*| (Pa); G_0*, |G*| at 30 ºC (Pa); G^*_{onset}, |G*| at the starch gelatinization onset (Pa); h, final height of dough (mm); H_m, maximum dough height (mm); H'_m, maximum height of gaseous production (mm); LV, Latent variables; OWA, optimum water absorption; Q^2, cumulative predictive variation from internal cross-validation; R^2, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error; T_1, time to reach the maximum dough height (min); T'_1, time to reach the maximum gas formation rate (min); tanδ, maximum tanδ; $tanδ_0$, tanδ at 30 ºC; TG*, temperature at maximum |G*| (ºC); Ttanδ, temperature at maximum tanδ (ºC);.

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4.4. Conclusions

Agroindustry by-products used in this study showed a potential application as a source of fibre additives to dough. Since a great variation of total dietary fibre was observed among recovered extracts, the addition had to be adjusted in order to produce breads that could be claimed as a “source of fibre” and “high in fibre”. The impact of wheat flour replacement on dough and bread characteristics varied with extract origin and replacement levels. In general, doughs and breads with 4% extract had less impact than higher extract concentrations. Therefore, it was possible to successfully obtain breads with the nutrition claim “source of fibre”. The impact of dietary fibre fortification on dough properties was proportional to the extract amount added, and consequently affected also bread parameters. Multivariate PLS regression highlights relevant information about the relationship between dough and bread data. In general, the use of dietary fibre recovered from agroindustry by-products is promising for food application with a potential functional role.

CHAPTER 5

Fortification wheat bread with agroindustry by-products: statistical methods for sensory preference evaluation and correlation with colour and crumb structure

Abstract

The use of agroindustry by-products (BP) for fortification of wheat bread can be an alternative to waste disposal since BP are appealing sources of dietary fibre. Moreover, it may also contribute to indirect income generation. In this study, sensory, colour, and crumb structure properties of breads fortified with fibre rich fraction recovered from four types of agroindustry BP were tested, namely orange (OE), pomegranate (PE), elderberry (EE) and spent yeast (YE). Statistical models for sensory preference evaluation and correlation with colour and crumb structure were developed.

External preference mapping indicated consumer preferences and enabled selection of the concentrations of BP fibre rich fraction with best acceptance, namely 7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE. Data collected from image analysis complemented sensory profile information, whereas multivariate PLS regression provided information on the relationship between “Crust colour” and “Crumb colour” and instrumental data. Regression models developed for both sensory attributes presented good fitting ($R^2Y > 0.700$) and predictive ability ($Q^2 > 0.500$), with low RMSE. Crust and crumb a* parameters had a positive influence on “Crust colour” and “Crumb colour” models, while crust L* and b* had a negative influence.

Key words: bread; agroindustry by-products; sensory analysis; colour; crumb structure; external preference mapping; partial least squares regression.

5.1. Introduction

By-products (BP) generated from food industry are interesting substrates for the extraction of bioactive compounds. Nevertheless, they usually present, per se, insufficient biological stability, high water content, and high enzymatic activity (23, 235-239, 261, 262). The recovery of bioactive compounds from food industry BP can be an alternative to waste disposal and may contribute to indirect income generation.

Orange is mainly consumed as a fresh fruit or as juice. The BP resulting from juice separation constitutes approximately 50% of the original fruit and includes peel (flavedo and albedo), pulp (juice sac residue), rag (membranes and cores) and seeds (17, 263). Orange BP is mainly composed by dietary fibre (DF), including pectin, cellulose, and hemicellulose and soluble sugars, namely glucose, fructose, and sucrose (16, 17). Pomegranate is consumed not only as fresh fruit but also as processed products, such as juices, jams, jellies, and extracts (264, 265). Pomegranate processing BP includes the peel and internal membranes and represents 50% of fresh fruit weight. Pomegranate peel has high quantity of proteins and DF, being also an important source of minerals (18-21). It also contains polyphenols and flavonoids (21, 266). Elderberry is used for juices, marmalades, wines, and syrups production (267-269). BP generated from elderberry processing contain skin, pulp, and seeds (pomace) and remains a good source of anthocyanins and other polyphenols, vitamins, and DF (22, 23).

Fruit BP are usually appealing sources dietary fibre. However, it should be noted that the nutritional value of plant foods can be reduced by the presence of some naturally occurring compounds, such as phytic acid that decreases mineral bioavailability, and tannins that reduce protein digestibility and present astringent properties (270, 271). Concerning pomegranate BP, condensed and ellagic tannins, gallic and ellagic acids are present in significant amounts (21, 266, 272) whereas, reported levels of phytic acid and tannins in orange and elderberry BP are either not detected or negligible (273, 274). Another interesting fibre source is spent yeast, which is the second major brewing BP. Yeast cell walls are rich in β-glucans, and a source of vitamins and cromium (25, 223).

Fortification of wheat bread with the above-mentioned agroindustry BP is an interesting possibility. Bread is consumed in a daily base; there is a growing interest in the incorporation of functional ingredients on bread to reply consumer’s demands on healthy nutrition (166-168). A disadvantage that might arise with bread addition of DF recovered from BP is the presence of compounds with bitter or astringent taste, or that impart darker colour, leading to lower preference in many consumers (275). As reported by Bakke and Vickers (276) sensory preferences can be an obstacle to non-refined bread consumption. Therefore, despite their health benefits, breads formulated with new functional ingredients must have high sensory acceptance in order to be selected and eaten. Otherwise, they will not be able to compete with traditional wheat bread. Colour and crumb structure are important attributes in bread and affect consumer preference. Instrumental acquired colour and crumb structure data can provide additional information and complement data obtained from sensory analysis. Statistical methods as External Preference Mapping are useful to examine individual consumers' acceptability and relate it to sensory, physical and/or chemical data (277). With this technique, based on Principal Component Analysis (PCA), cluster and polynomial regression, it is possible to take into consideration heterogeneity in acceptability among consumers (278, 279). Partial Least Squares (PLS) regression is another valuable method for analysing or predicting a set of dependent variables from a set of independent variables (predictors). The combination of PCA with multiple regression allows the extraction, from the predictors, of a set factors (latent variables) exhibiting the best predictive ability (280). Therefore, statistical methods can be useful tools for sensory preference evaluation and correlation with colour and crumb structure.

Sensory, colour, and crumb structure properties of breads with increased fibre content recovered from agroindustry BP were studied. Four types of agroindustry BP that are potential sources of DF were tested: orange peel (OBP), pomegranate peel and interior membranes (PBP), elderberry skin, pulp and seeds (EBP) and spent yeast (YBP). The major goal of this study is to select the concentrations of fibre rich extracts with best acceptance by consumers and understand the relationships between sensory and instrumental data, for this purpose a combined mathematical modelling approach to study bread profile analysis and preference, as well as to evaluate colour and crumb structure relationship with sensory profile was carried out.

5.2. Material and methods

5.2.1. Fibre rich fraction from agroindustry by-products

Agroindustry by-products used in this study are mainly composed by dietary fibre (23, 235-239), however, it is necessary to recover fibre fraction while inactivating existing enzymes. Oranges and elderberries were supplied by local producers, and pomegranates were purchase in a local market in Porto, Portugal. Oranges and pomegranates were washed and split into halves. Orange peel (OBP), and pomegranate peel and interior membranes (PBP) were frozen, and afterwards roughly grinded in a Knife Mill Grindomix GM200 (Retsch GmbH, Germany). For the concentration of fibre rich fraction from OBP, 1000 g sample was heated with deionized water at 97 ºC (solid/water ratio 1:10, w/v) for 10 min. Afterwards, the residue was collected with a strainer, dried in a dynamic oven at 70 ºC for 24 h, and grinded into a fine powder. Subsequently, the powder was extracted with 95%

ethanol (solid/ethanol ratio 1:4, w/v), centrifuged at 4500 *g* for 10 min and the remaining residue was re-extracted under same conditions, and washed two times with deionized water. The final extracted residue (OE) was dried in a dynamic oven at 70 ºC for 24 h. Concerning extraction of fibre fraction from PBP the process consisted in heating 1000 g sample with deionized water at 97 ºC (solid/water ratio 1:10, w/v) for 10 min. The extracted solid residue (PE) was collected with a strainer, dried in a dynamic oven at 70 ºC for 24 h. Elderberries were freeze dried, and 1000 g of sample was macerated with 1250 mL of 95% ethanol. Supernatant was collected, and maceration procedure repeated three times. The supernatant collected from this procedure can have application as colorant, due to the high content of anthocyanins. Remaining elderberry skin, pulp and seeds (EBP) was washed with distilled water (solid/water ratio 1:10, w/v). The mixture was centrifuged at 4500 *g* for 10 min and the pellet was re-extracted under same conditions. The final extracted solid residue (EE) was dried in a dynamic oven at 40 ºC for 24 h. With respect to spent yeast biomass (YBP) from Lager beer (*Saccharomyces pastorianus*) production it was supplied by a local beer industry. The extraction procedure applied to YBP, in order to obtain a fibre rich fraction, was carried out as described by Thammakiti *et al.* (204) and the yeast residue (YE) was freeze-dried (204). After being oven/freeze-dried, solid residues (extracts) obtained from every BP were grinded into a fine powder and stored in a desiccator at room temperature until further use.

5.2.2. Bread samples

Breads were made using home-making bread machines (Moulinex, Groupe SEB, France). The selected bread making program included dough preparation (2h20m) and baking (20 min). After baking, every bread was cooled at room temperature during 90 min before further analyses. Wheat flour, flour improver, fresh yeast, and salt, supplied by Forno de Espinho bakery (Espinho, Portugal) were used to carry out this study. Breads were prepared following the bakery recipe: 400 g wheat flour, 4.0 g of flour improver, 8.0 g fresh yeast, 5.2 g salt, and 240.0 g water. Nine different bread formulations were produced with addition of fibre rich extracts (% wheat flour): EE (5.0%, 7.0%, 10.0%), OE (2.5%, 7.0), PE (5.0%, 7.0%, 10.0%), YE (2.5%). For control (C) bread no extract was added. Considering existing EFSA recommendations on yeast β-glucans, with an upper threshold set at 200 mg yeast β-glucans per serving (281), its content was determined in YE using an Enzymatic Yeast β-Glucan kit (K-EBHLG, Megazyme International Ireland Ltd., Ireland. No YE values greater than 2.5% were tested, as it would be above the threshold regarded as safe (281).

5.2.3. Sensory analysis

5.2.3.1. Sensory profile of bread samples by a trained panel

Sensory profile was evaluated in order to understand the influence of addition of fibre rich extracts on bread sensory characteristics. A sensory panel composed by 13 members was trained for descriptive analysis according to the guidelines in the ISO 8586 (185). In the training sessions, a list of attributes was suggested by panellists and redundant descriptive terms were removed after tasting control bread samples (186). A score card was developed to evaluate attribute intensities on 8.5-cm unstructured line scales (minimum intensity - 1; maximum intensity - 7) and anchors were defined for selected attributes. The score card was tested by the panel using unknown representative samples. A final list of 17 attributes was selected for bread descriptive profile: 5 overall appearance related attributes ("Crust colour", "Crumb colour", "Cell size", "Homogeneity", "Moist"), 3 odour related attributes ("Yeast", "Secondary odour", "Odour intensity"), 7 aroma related attributes ("Salty", "Sour", Bitter", "Astringent", "Secondary", "Aftertaste", "Aroma intensity"), and 2 texture related attributes ("Crunchy crust", "Cohesiveness").

In evaluation sessions, bread slices (1.5 cm thickness), including the crust and crumb, were presented to assessors in a 3-digit coded glass covered with a glass lid. Assessment was carried out individually under white light at room temperature. Each assessor was provided with filtered water and asked to cleanse their palate between tastings. Control and fibre rich breads replicates were analysed over eight sessions.

5.2.3.2. Consumer acceptance of bread samples

Sensory acceptability tests were carried out, with University of Porto undergraduates, graduates and employees, who showed interest in participating on the bread sensory test.

Bread samples were served in plastic plates in a randomized manner. Sixty consumers participated in the trial; they were aged between 20 and 50 year and were regular consumers of bread (at least once a day). Acceptance testing was conducted using a 7-point hedonic scale (7 = like extremely, 1 = dislike extremely) to assess the overall acceptability.

5.2.4. Crumb structure image analysis

Crumb structure was evaluated by image analysis. Five slices (1.5 cm thickness) from three breads of every formulation were cut and analysed. Each slice was positioned on the flatbed scanner (Canon iR2016i, Netherlands) in standardized conditions (black cardboard box over the slice, in order enhance contrast) (206). Images were captured in the RGB (24 bit) standard format with a resolution of 300 dpi and saved in TIFF format. Each image was processed and analysed using code written in Matlab R2015a (MathWorks). A single 500x500 pixel (42.3x42.3 mm) field of view (FOV) was cropped from the image centre and converted to a 256 level grey scale. Image segmentation was performed with Otsu's method and cell morphological parameters were analysed with Image Processing Toolbox 9.4 from Matlab (207). Data resulting from the crumb structure analysis included: number of cells, mean cell area (mm^2), and cell density ($cells/mm^2$). Additionally, cells were separated into different classes as a function of their area: very small size ($0.2\ mm^2 \leq$ cell area); small size ($0.2 <$ cell area $\leq 3.0\ mm^2$); medium size ($3.0 <$ cell area $\leq 10.0\ mm^2$); large size (cell area $> 10.0\ mm^2$).

5.2.5. Evaluation of bread colour

Colour analysis was achieved by two different approaches: colorimeter and image analysis. For the first approach, three breads of every formulation were cut in half and the measurements were performed in three different points on the bread crumb and side crust. A Minolta CR-300 colorimeter (Minolta, Ramsey, NJ) with illuminate D65, a 0° standard observer and a 2.5 cm port/viewing area was used for measurement of colour in the CIElab system (colour represented in the L^*, a^*, b^* space). L^* represents the luminance or lightness component, with values from 0 (black) to 100 (white). The two chromatic components are a^* and b^*. In a^*, negative values are green and positive are red, and in b^* negative values are blue and positive are yellow (282). The colorimeter was standardized with a white tile having the following values: $L^* = 93.5$, $a^* = 1.0$ and $b^* = 0.8$ before measuring crust and crumb colour on each bread. For the second approach, each single 500x500 pixel FOV obtained from bread image analysis (described in previous section) was converted from RGB to CIElab system using code written in Matlab R2015a (MathWorks).

5.2.6. Statistical analysis

Selection of the concentration of fibre rich extract that gives best bread sensory performance, measured as consumers overall acceptability, was carried out for each agroindustry BP studied. Sensory data collected was treated using External Preference Mapping technique (277, 279, 283).

Having set the different concentrations of fibre rich extract from each agroindustry BP, differences between bread with EE, OE, PE, and YE addition, and C bread were evaluated. All dependent variables from every bread formulation were tested for the residuals distribution with Shapiro–Wilk's test. One-way ANOVA or Kruskal–Wallis test were applied, according to the residuals distribution. Whenever statistical significances were found with one-way ANOVA, Tukey´s or Tamhane's T2 post-hoc tests were applied for mean comparison, depending on equal variances assumption or not. When statistical significances were found with Kruskal–Wallis test, Dunn's test post-hoc test was applied for median comparison.

PLS regression was also used to study the relationships between sensory attributes (Y-matrix) and colour, and crumb structure (X-matrix) in terms of prediction of Y-variables from X-variables Random validation was also applied to identify relevant X-variables. Scores and loading plots were analysed, as well as, calibration and validation coefficients.

All statistical analyses were conducted with the XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France) at 10% (Preference mapping) and 5% (ANOVA, Kruskal–Wallis, and PLS regression) significance level.

5.3. Results and discussion

5.3.1. Sensory analysis

5.3.1.1. External Preference Mapping

External preference mapping includes three sequential steps: creates the sensory map, groups the consumers, and creates the preference map using the PREFMAP method. To create the sensory map, a Principal Component Analysis (PCA) was applied on sensory data of attributes evaluated by the trained panel. Then, using Agglomerative Hierarchical Clustering (AHC), the consumers were grouped into homogeneous groups according to their preference (overall acceptability). Finally, PREFMAP method was employed using the sensory attribute coordinates in the two-dimensional factor space (explaining 73.12% of existing variability), resulting from PCA, and average overall acceptability scores for each of the 3 clusters, obtained from AHC. In this method, four different regression models were tested to predict each consumer group overall acceptability: vector model, circular model, elliptical model, and quadratic surface model.

The resulting preference map (see Figure 5.1) indicated the best fitting model for each cluster and consumers preference. The vector model was the best fit ($p < 0.05$) for cluster 1 (C1), and elliptical model was the best for clusters 2 and 3 (C2 and C3) but only significant ($p < 0.05$) for C3. In C1, the vector indicated the direction in the map where overall acceptability increased. In this cluster, the preference order was C > 2.5% YE > 2.5% OE > 5.0% PE > 7.0% PE > 7.0% EE > 5.0% OE > 5.0% EE > 10.0% EE > 10.0% PE. The elliptical model for C2 showed a saddle point, where the thicker lines indicated the direction in which overall acceptability increased, and the thinner ones to the direction in which it decreased. Here, the preference order was 5.0% OE > 5.0% EE > 5.0% PE > 7.0% EE > 7.0% PE > 10.0% EE > 2.5% OE > 2.5% YE > C > 10.0% PE. In C3, the elliptical model signalled the ideal point, indicating the location where overall acceptability was higher. For this cluster, the preference order was 5.0% PE > 7.0% EE > 7.0% PE > C > 2.5% OE > 2.5% YE > 10.0% PE > 5.0% EE > 10.0% EE > 5.0% OE.

Regarding to consumers satisfaction for each bread formulation, 2.5 % OE and 5.0% PE were the breads with higher preference (approved by every consumer), followed by C, 7.0% EE, 7.0% PE, and 2.5% YE breads, which were approved by 67% of the consumers. Lower preference was found for 5.0% EE, 10.0% EE, and 5.0% OE breads (33%), and the least preferred bread was 10.0% PE (0%).

Considering information collected from the external preference mapping, the selected concentrations for further evaluation were: 7.0% EE; 2.5% OE; 5.0 %, PE; 2.5% YE. As overall acceptability scores for 2.5% YE bread were positive and exhibited a preference similar to C bread, thus, reduction of its concentration was not necessary.

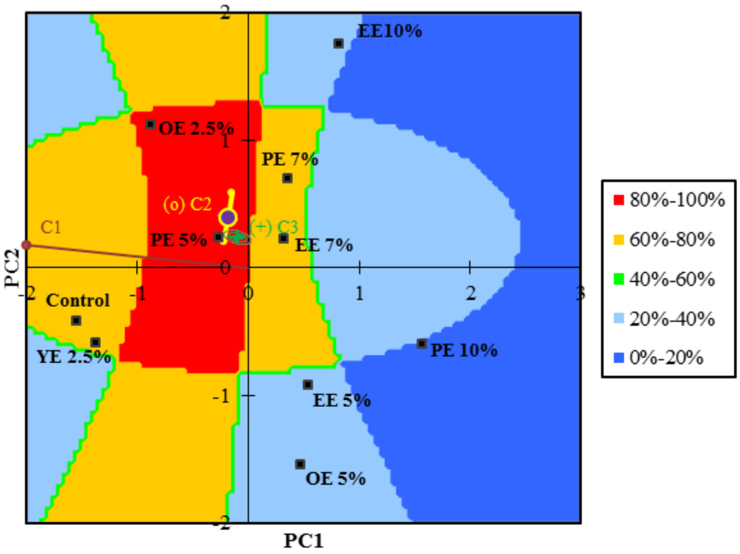

Figure 5.1. Preference mapping showing 3 clusters: 1 (vector), 2 (elliptical (o); where the thicker lines corresponds to the direction in which the preference increases, and the thinner ones to the direction in which it diminishes) and 3 (elliptical (+); where the plus indicates a maximum point in terms of overall acceptability); and the 5 regions of the global average acceptance.

C1, Cluster 1; C2, Cluster 2; C3, Cluster 3; EE, Elderberry extract; OE, Orange extract; PE, Pomegranate extract; YE, Yeast extract.

5.3.1.2. Sensory comparison of selected breads

Median sensory scores for C, 7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE breads, one-way ANOVA and Kruskal–Wallis results are provided in Table 5.1. In overall appearance, both “Crust colour” and “Crumb colour” had significantly ($p < 0.05$) higher scores (darker colour) for 7.0% EE and 5.0% PE breads. “Cell size” scores were significantly ($p < 0.05$) higher (bigger cells) for 7.0% EE, 2.5% OE, and 5.0% PE breads. In “Moist”, 5.0% PE bread was the only with significant differences ($p < 0.05$) from C bread. Regarding odour, aroma, and texture attributes, significantly ($p < 0.05$) higher scores were found for 7.0% EE and 5.0% PE breads, except “Yeast”, “Salty”, and “Cohesiveness”. Higher scores in aroma attributes could be due to phenolic compounds present in EE and PE (269, 284).

Table 5.1. Values for descriptive sensory analysis for bread with different extract addition levels (% wheat flour), on dry weight basis

Attribute	**Extract (% wheat flour)**					***p***
	C (0.0)	EE (7.0)	OE (2.5)	PE (5.0)	YE (2.5)	
Overall appearance						
Crust colour	1.99^{a} (1.49–2.98)	5.59^{b} (3.22–7.00)	1.99^{a} (1.21–3.15)	3.30^{b} (2.16–5.52)	2.11^{a} (1.92–2.98)	$<0.001^{***}$
Crumb colour	1.99^{a} (1.67–2.45)	6.14^{b} (4.18–7.00)	2.34^{a} (1.64–3.47)	3.97^{b}(2.45–5.38)	2.11^{a} (1.60–3.33)	$<0.001^{***}$
Cell size	5.02^{a} (2.55–5.91)	5.29^{b} (4.21–6.33)	5.28^{b} (3.54–6.22)	5.42^{b} (3.33–6.36)	4.92^{a} (3.26–5.59)	$<0.001^{***}$
Homogeneity	4.16 (2.80–5.59)	3.69 (1.92–5.45)	4.00 (2.66–6.12)	3.67 (2.59–5.98)	3.83 (2.62–5.20)	ns
Moist	3.01^{a} (2.76–3.61)	3.62^{ab} (1.92–5.41)	3.40^{ab} (1.64–6.15)	4.06^{b} (1.74–5.55)	3.01^{a} (1.95–4.46)	0.001^{**}
Odour						
Yeast	4.00 (1.00–5.59)	3.37 (1.25–4.74)	3.75 (1.00–5.59)	3.90 (1.00–5.31)	3.97 (2.87–4.95)	ns
Secondary odour	1.18^{a} (1.00–1.56)	3.63^{b} (1.18–6.22)	1.32^{a} (1.18–4.32)	2.33^{b} (1.28–4.78)	1.27^{a} (1.04–2.27)	$<0.001^{***}$
Odour intensity	3.01^{a} (2.02–3.68)	4.23^{b} (2.27–6.01)	3.12^{ab} (1.92–5.45)	3.89^{b} (2.02–4.78)	3.01^{a} (2.73–3.72)	$<0.001^{***}$
Aroma						
Salty	1.99^{ab} (1.49–2.84)	1.99^{ab} (1.07–3.08)	2.22^{b} (1.14–4.42)	2.03^{ab} (1.07–4.14)	1.99^{a} (1.11–2.41)	0.049^{***}
Sour	1.28^{a} (1.14–1.53)	1.95^{b} (1.07–4.18)	1.28^{a} (1.00–2.34)	1.56^{bc} (1.00–4.21)	1.39^{ac} (1.07–1.92)	$<0.001^{***}$
Bitter	1.99^{a} (1.92–2.48)	2.87^{b} (1.67–4.32)	1.99^{ac} (1.14–2.91)	2.34^{bc} (1.07–3.75)	2.13^{ac} (1.04–2.93)	$<0.001^{***}$
Astringent	1.99^{a} (1.00–2.98)	2.45^{b} (1.00–4.14)	1.99^{abc} (1.00–2.87)	2.29^{bc} (1.07–4.04)	1.99^{ac} (1.07–3.68)	0.001^{***}
Secondary	1.28^{a} (1.00–2.24)	2.78^{b} (1.00–5.45)	1.37^{a} (1.00–2.98)	2.04^{b} (1.28–3.93)	1.28^{a} (1.07–2.52)	$<0.001^{***}$
Aftertaste	1.28^{a} (1.14–2.69)	2.15^{b} (1.07–4.81)	1.60^{abc} (1.07–4.07)	1.66^{bc} (1.07–3.75)	1.30^{ac} (1.85–2.73)	$<0.001^{***}$
Aroma intensity	2.89 ± 0.61^{a}	4.12 ± 1.00^{b}	3.25 ± 0.76^{ac}	3.68 ± 0.78^{bc}	2.95 ± 0.57^{a}	$<0.001^{**}$
Texture						
Crunchy crust	3.35 ± 0.93^{a}	4.10 ± 0.91^{b}	3.22 ± 0.90^{a}	4.90 ± 0.70^{c}	3.67 ± 0.86^{ab}	$<0.001^{*}$
Cohesiveness	3.01 (1.64–4.53)	3.22 (2.20–4.88)	3.22 (1.67–5.87)	3.35 (1.99–5.34)	3.01 (2.24–3.96)	ns

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=13).

C, Control; EE, Elderberry extract; OE, Orange extract; ns., not significant; PE, Pomegranate extract; YE, Yeast extract.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

[*] *p* Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

[**] *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

[***] *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test.

5.3.2. Crumb structure image analysis

Crumb morphological features of the different bread formulations are compared in Table 5.2. Crumb morphology is an important bread quality parameter alongside taste, crumb colour and crumb texture (168, 209). One-way ANOVA and Kruskal–Wallis revealed differences in morphological features for every bread formulation. Bread with 7.0% EE was the one with significantly ($p < 0.05$) higher number of cells and cell density, and with lowest mean area. The opposite was registed for 2.5% OE bread. When observing cell area distribution, more than 80% of cells in every bread formulation had an area below 3.0 mm^2. Comparing to C bread, 7.0% EE bread had signifficantly ($p < 0.05$) higher percentage of cells pertaining to very small size class (0.2 mm^2 ≤ cell area) and lower cells distrubuted in medium (3.0 mm^2 < cell area ≤ 10.0 mm^2) and large (cell area > 10.0 mm^2) size classes.

When image analysis was compared with sensory profile, the sensory panel was not able to identify which bread formulation had larger or smaller cells. Data gathered from image analysis provided information that would not be possible to obtain from sensory data.

5.3.3. Bread colour

Crust and crumb colour of different bread formulations varied considerably (Table 5.2). Bread colour depends on factors such as formulation or baking conditions. Maillard reactions and crust caramelization are responsible for brown colour formation, but it can be masked by the colour imparted through the ingredients used in bread formulation. Bread image analysis, rather than colorimeter, has been used in some studies for colour data collection (27, 285, 286). In this study, colour data acquisition was done with colorimeter and image analysis, and both methods were compared for bread crumb.

In relation to bread crust colour parameters (measured with colorimeter), crust L* was significantly ($p < 0.05$) lower (darker) than C bread in 7.0% EE and 5.0% PE breads. While C, 2.5% OE, and 2.5% YE breads had a greenish crust colour (negative a* values), 7.0% EE and 5.0% PE showed a reddish crust colour (positive a* values). Bread crust exhibited a yellowish colour (positive b* values) for every bread formulation, but significantly higher than C bread for 2.5% OE and 5.0% PE as observed in Table 5.2.

Regarding to bread crumb colour parameters, some differences were found between measurements done with colorimeter versus image analysis. Colorimeter crumb L* values for 7.0% EE and 5% PE bread were significantly ($p < 0.05$) lower than C bread, while image analysis values were only lower ($p < 0.05$) for 7.0% EE bread. 7.0% EE and 5.0% PE bread crumbs were redder ($p < 0.05$) than C bread in both methods. On image analysis was possible to distinguish 2.5% OE bread crumb a* from C bread. Image analysis was able to distinguish ($p < 0.05$) C bread crumb b* from 7.0% EE (lower), 2.5% OE (higher), and 5.0%

PE (higher), while colorimeter only revealed differences for 7.0% EE. Variation found between colorimeter and image analysis could be due to the number of points analysed by replicate; while 3 points with approximately 2 cm^2 were analysed per replicate in colorimeter measurements, an average of 250 000 pixels were considered in image analysis, which could be more accurate (282).

Modelling relationships between bread crumb colour measured with colorimeter and image analysis were also studied. Colorimeter crumb colour data was successfully fitted from data obtained with image analysis through nonlinear regressions. Colorimeter L* and a* could be obtained from image analysis using a four-degree polynomial regression (r^2 = 0.852, RMSE = 7.059; r^2 = 0.790, RMSE = 1.801, respectively), whereas b* could obtained using a two-degree polynomial regression (r^2 = 0.923, RMSE = 1.428). So, image analysis is an interesting alternative to the colorimeter.

Additionally, comparing bread crust and crumb colour results with the ones obtained from sensory profile, it was possible to observe that the panel was able to identify breads with darker crust and crumb (7.0% EE and 5.0% PE). Also, the sensory panel did not find differences incrust and crumb lightness between C bread, and 2.5% YE and 2.5% OE breads, which was in agreement with data collected from colorimeter and image analysis.

Table 5.2. Values for colour parameters and crumb image analysis for bread with different extract addition levels (% wheat flour), on dry weight basis.

Colour	Extract (% wheat flour)					p
	C (0.0)	EE (7.0)	OE (2.5)	PE (5.0)	YE (2.5)	
Number of cells	529^{ab} (368–569)	710^{c} (553–889)	427^{a} (359–580)	548^{b} (453–863)	487^{ab} (359–677)	$<0.001^{***}$
Mean area (mm^2)	6.09^{ab} (4.65–9.22)	4.70^{c} (3.79–6.00)	8.01^{a} (5.99–9.54)	6.01^{bc} (4.03–7.48)	7.00^{ab} (4.98–9.65)	$<0.001^{***}$
Cell density (cells/mm^2)	88.00^{ab} (39.92–156.57)	150.48^{c} (92.14–234.31)	53.34^{a} (37.64–96.76)	90.05^{bc} (60.57–214.41)	69.55^{ab} (37.30–135.90)	$<0.001^{***}$
Cell area						
(% of total cells)						
$0.2 \le CA$ (mm^2)	58.50 ± 2.50^{a}	66.45 ± 2.94^{b}	58.16 ± 3.53^{a}	60.49 ± 2.42^{a}	57.65 ± 2.50^{a}	$<0.001^{*}$
$0.2 < CA \le 3.0$ (mm^2)	30.70^{ab} (27.51–34.94)	30.33^{a} (23.47–32.38)	30.35^{ab} (25.27–34.75)	31.16^{ab} (29.05–35.22)	33.55^{b} (28.48–35.43)	0.045^{***}
$3.0 < CA \le 10.0$ (mm^2)	4.69 ± 1.25^{ab}	2.25 ± 0.75^{c}	4.98 ± 0.79^{a}	4.04 ± 0.81^{b}	4.63 ± 1.42^{ab}	$<0.001^{**}$
$CA > 10.0$ (mm^2)	5.16^{ab} (4.92–8.12)	1.82^{c} (0.80–3.04)	6.82^{a} (4.19–8.67)	3.80^{bc} (2.94–6.22)	5.39^{ab} (3.40–8.08)	$<0.001^{***}$
Crust						
L*	62.79^{a} (57.09–72.01)	25.38^{b} (21.94–31.56)	60.37^{a} (55.12–64.88)	47.66^{b} (44.16–56.16)	65.06^{a} (57.29–69.85)	$<0.001^{***}$
a*	-0.55^{a} (-0.84–0.45)	8.96^{b} (7.34–10.99)	-0.73^{a} (-1.31–0.02)	6.01^{b} (5.47–6.80)	-0.42^{a} (-0.65–0.08)	$<0.001^{***}$
b*	10.62^{ab} (9.47–15.80)	3.49^{a} (2.76–4.19)	15.68^{cd} (12.65–19.03)	20.04^{d} (18.63–21.16)	13.58^{bc} (10.16–16.53)	0.001^{***}
Crumb						
L*	76.32^{a} (72.32–77.99)	36.48^{b} (24.16–45.23)	74.88^{a} (55.71–79.34)	58.23^{b} (41.89–67.68)	72.23^{a} (57.90–79.13)	$<0.001^{***}$
$L^{*\,(i)}$	39.97^{ab} (33.98–44.37)	19.06^{c} (16.32–20.02)	36.84^{ab} (29.31–43.51)	36.82^{a} (33.70–39.19)	40.79^{b} (34.41–44.36)	$<0.001^{***}$
a*	2.98^{a} (2.15–4.56)	11.90^{b} (8.35–14.69)	3.29^{a} (1.73–5.34)	6.12^{c} (3.38–7.75)	4.40^{ac} (2.15–7.93)	0.001^{***}
$a^{*\,(i)}$	2.59 ± 0.42^{a}	8.54 ± 0.33^{b}	3.13 ± 0.51^{c}	8.86 ± 0.42^{b}	2.79 ± 0.40^{ac}	$<0.001^{*}$
b*	20.52 ± 1.77^{a}	10.02 ± 2.48^{b}	21.20 ± 2.44^{a}	20.21 ± 3.34^{a}	22.11 ± 3.85^{a}	$<0.001^{*}$
$b^{*(i)}$	14.43 ± 0.99^{a}	1.89 ± 0.17^{b}	18.57 ± 2.35^{c}	13.92 ± 0.68^{a}	15.83 ± 2.00^{d}	$<0.001^{**}$

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=18, n=15, respectively).

[(i)] Values obtained from image analysis.

C, Control; CA, Cell area; EE, Elderberry extract; OE, Orange extract; PE, Pomegranate extract; YE, Yeast extract.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test

5.3.4. Correlation of sensory characteristics with colour and crumb structure

Multivariate PLS regression was performed in order to study the correlation of sensory data with instrumental (colour and crumb structure) data, based on sensory data prediction (Y-variables) from instrumental data (X-variables). Table 5.3 summarizes sensory attributes prediction (collectively and individually) from instrumental parameters. An effective regression model, with good predictive ability could indicate an influence of colour and crumb structure on sensory analysis profile, regardless the agroindustry BP used.

Of the 17 sensory attributes analysed, 14 were found to be correlated with instrumental parameters measured. Therefore, “Homogeneity”, “Yeast”, and “Cohesiveness” were not included. In order to obtain a successful regression model, R^2X and R^2Y had to be equal or superior to 0.700. The ability to predict new samples was evaluated by Q^2 that should be equal or superior to 0.500 (259). Collectively, although the obtained regression model successfully fitted X-variables (colour and crumb structure) data, with an $R^2X = 0.837$, it poorly fitted the Y-variables (sensory attributes) data ($R^2Y < 0.500$). As a consequence of the poor Y-variables data fitting, the model predictive ability was also poor ($0.000 < Q^2 < 0.500$). Analysing Y-variables individual response on the model, it was possible to observe that only “Crust colour” and “Crumb colour” were well fitted ($R^2Y > 0.700$), with good predictive ability ($Q^2 > 0.500$), and lowest RMSE values. Remaining sensory attributes were poorly fitted ($R^2Y < 0.500$), with poor ($0.000 < Q^2 < 0.500$) or lacking ($Q^2 < 0.000$) predictive ability, and higher RMSE.

The relationship between sensory attributes “Crust colour” and “Crumb colour”, and instrumental colour data was further explored with univariate PLS regression (see Figure 5.2 a and b). Regression models developed for both sensory attributes presented good fitting ($R^2Y > 0.700$) and predictive ability ($Q^2 > 0.500$), with low RMSE. The importance of colour parameters in the projection and their standardized coefficients was also determined, and latent variables were identified (see Figure 2 c and d). Every colour parameter was a latent variable, except b* crust. Crust and crumb a* parameters had a positive influence on “Crust colour” and “Crumb colour” models, while crust L* and b* had a negative influence. This could suggest that panel perception of bread darkness could be not only related to lower L*, but also with redness (higher a*) and blueness (lower b*).

Table 5.3. Results of multivariate PLS regression between colour parameters and crumb image analysis (X-variables) and bread parameters (Y-variables).

Sensory attributes	R^2X	R^2Y	Q^2	Sensory attributes	R^2Y	Q^2	RMSE
Collectively				*Individually*			
Crust colour; Crumb colour; Cell size; Moist; Secondary odour; Odour intensity; Salty; Sour; Bitter; Astringent; Secondary; Aftertaste; Aroma intensity; Crunchy crust;	0.837	0.367	0.175	Crust colour	0.739	0.633	0.414
				Crumb colour	0.900	0.862	0.254
				Cell size	0.098	-0.351	0.886
				Moist	0.325	-0.074	0.758
				Secondary odour	0.481	0.252	0.599
				Odour intensity	0.307	0.094	0.765
				Salty	0.053	-0.277	0.891
				Sour	0.293	-0.032	0.804
				Bitter	0.363	-0.082	0.776
				Astringent	0.182	-0.236	0.755
				Secondary	0.454	0.195	0.665
				Aftertaste	0.272	-0.085	0.853
				Aroma intensity	0.166	-0.117	0.815
				Crunchy crust	0.498	0.346	0.773

Q^2, cumulative predictive variation from internal cross-validation; R^2X, cumulative explained variation of X explained in terms of sum of squares; R^2Y, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error

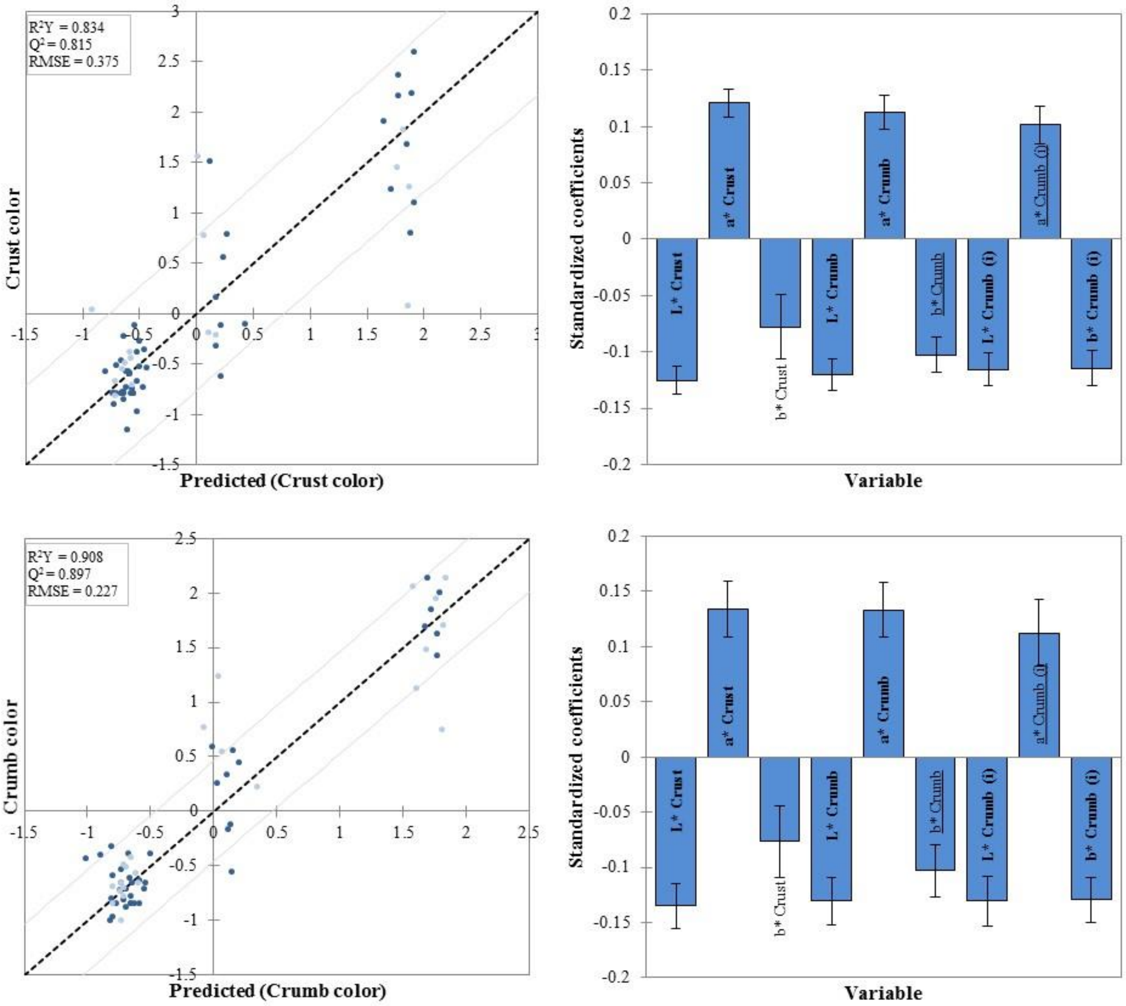

Figure 5.2. (a) Predicted versus actual standardized scores and **(b)** estimated standardized regression coefficient, for selected sensory attributes: "Crust colour" (1), and "Crumb colour" (2), modelled by PLS with 95% confidence interval. Highly influential latent variables (Variable Importance for the Projection > 1) are represented in bold and the moderately influential latent variables (0.8 < Variable Importance for the Projection < 1) are underlined. Actual values are represented by ● and predicted values by ○.

CA, Cell area; Q^2, cumulative predictive variation from internal cross-validation; R^2, cumulative explained variation of Y explained in terms of sum of squares.

5.4. Conclusions

Bread sensory characteristics were influenced by the addition of fibre rich fraction from different agroindustry BP. Mathematical modelling was shown as a relevant tool to study bread acceptability and understand relationships between sensory and instrumental data. External preference mapping was appropriate to study consumer preferences and to select the concentrations of fibre rich extracts with best acceptance, namely 7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE. Nevertheless, bread formulations with 7.0% EE and 5.0% PE registered more differences from C bread on colour and crumb structure than 2.5% OE and 2.5% YE. Data collected from image analysis complemented sensory profile information. Image analysis could be an interesting alternative to the colorimeter, since crumb colour data measured by colorimeter was successfully fitted from data obtained with image analysis. Additionally, multivariate PLS regression provided information on the relationship between sensory and instrumental data (colour and crumb structure). Successful modells were only obtained for “Crust colour” and “Crumb colour”. However, these relationships should be interpreted as associations and not as direct cause and effect, once observed correlations do not necessarily imply causality. Further studies will be performed to evaluate rheological properties and nutritional characterization of those wheat breads fortified with agroindustry BP.

PART IV

AGROINDUSTRY BY-PRODUCTS INFLUENCE ON MINERAL BIOACCESSABILITY

CHAPTER 6

Fibre fortification of wheat bread: Impact on mineral composition and bioaccessibility

Abstract

In this work, wheat bread was fortified with fibre enriched extracts recovered from agroindustry by-products, namely, elderberry skin, pulp and seeds (EE); orange peel (OE); pomegranate peel and interior membranes (PE); and spent yeast (YE). The impact of this fortification on total and bioaccessible mineral composition of wheat breads, estimated mineral daily intake, and the relationship between bioaccessibility and dietary fibre was evaluated.

Fortification with OE, EE, and PE improved the bread content on essential minerals when compared to control bread. The exception was bread fortified with YE, which presented a mineral content similar to control bread, but its mineral bioaccessibility was significantly higher than in all the other bread formulations. The opposite was observed for PE bread, which presented a significant reduction of bioaccessible minerals. We concluded that the origin of fibre rich extract must be carefully selected, to avoid potential negative impact on mineral bioaccessibility.

Keywords: bread, agroindustry by-products, mineral, bioaccessibility

6.1. Introduction

Micronutrients play a key role in maintaining body functions, namely the so-called "essential elements" (macrominerals and several trace elements). Major essential minerals, i.e., sodium (Na), calcium (Ca), potassium (K), and magnesium (Mg), and trace elements such as manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo), are important to body homeostasis (287). Na is necessary to maintain the volume of extracellular fluid and plasma osmolality, while Ca is important for bone metabolism, vascular, neuromuscular, and glandular functions. K is the main intracellular cation and it is essential for normal cellular function. Mg is a cofactor required for over 300 enzyme systems, it is also involved in bone metabolism and preservation of intracellular levels of K and Ca. Mn is necessary for bone formation and in metabolism of amino acids, cholesterol, and carbohydrates. Fe is a key component in a wide variety of proteins, including hemoglobin and several enzymes. Cu is also a cofactor of several enzymes, responsible for the reduction of molecular oxygen. Zn has been shown to be essential for growth and development, exhibiting catalytic, structural, and regulatory biological functions. It is a cofactor required for more than 200 enzymes. Se, is a component of the antioxidant enzyme glutathione peroxidase (GPx), plays an important role in the defence against oxidative stress. Mo has been shown to act as a cofactor for several enzymes, comprising sulphite oxidase, xanthine oxidase, and aldehyde oxidase (287, 288).

Adequate minerals intake does not necessarily mean adequate systemic absorption, since this depends on their bioaccessibility and bioavailability (289, 290). With respect to bioavailability, it should be determined by human or animal *in vivo* measurements. Nevertheless, valuable information on minerals availability can be provided by *in vitro* methods that simulate gastrointestinal digestion and minerals release in the gastrointestinal tract, which are then available for absorption (bioaccessibility) (289, 290). These methods are widely used due to their good correlation with *in vivo* studies (291, 292).

Dietary fibre (DF) also plays an important role in human diet and health, namely on the regulation of intestinal transit time and visceral hypersensitivity, decrease on several types of cancer incidence, prevention and treatment of hypertension, cardiovascular diseases and diabetes (293). DF extracted from agroindustry by-products (BP), namely fruits and brewing, also contains several minerals and can be used as an ingredient in a wide variety of food products of regular consumption (19, 25, 58, 223).

Bread is one of the most widely consumed foods and an important source of nutrients, such as proteins, dietary fibre, starch, vitamins, and minerals. According to World Health Organization (WHO) dietary guidelines, in European countries, bread consumption recommendation is around 250 g of bread per person per day (294). Although bread consumption significantly varies according to cultural habits, bread typical daily intake makes it an interesting vehicle for DF and mineral enrichment through extracts recovered from fruit and brewing BP (19, 61, 127, 166, 168). The increase of DF content may have a positive impact in human health, but it can also reduce bioaccessibility of major minerals and trace elements (295-297). Additionally, other anti-nutrients can be co-extracted from BP, namely oxalic acid, phytic acid, and tannins. These naturally occurring compounds act as mineral binders or chelators, resulting in insoluble salts, or very poorly dissociated chelates (287, 298).

In this work, wheat bread was fortified with different fibre enriched extracts recovered from agroindustry BP: elderberry skin, pulp and seeds (EE); orange peel (OE); pomegranate peel and interior membranes (PE); and spent yeast (YE). The main goals of this study were to understand the impact of wheat bread fortification with fibre rich extracts on mineral composition, bioaccessibility, and estimated mineral daily intake.

6.2. Experimental

6.2.1. Raw materials and extraction procedures

Agroindustry BP, from orange, elderberry, pomegranate, and spent yeast contain dietary fibre (23, 235-239) with different composition of soluble and insoluble fibre. Fibre fraction to be used as food additive requires inactivation of existing enzymes and reduction of compounds that can have a negative impact on sensory characteristics (i.e. phytic acid and tannins). Thus, due to the different composition of the by-products used (23, 235-239), the procedures to obtain fibre enriched extracts must be adapted for each BP.

Edible oranges and elderberries were supplied by local producers, and pomegranates were purchase in a local market in Porto, Portugal. The spent yeast biomass was extracted from Ale beer production and supplied by a local beer industry. Yeast extract (YE) was obtained from spent yeast through autolysis and lyophilized, as described by Thammakiti *et al.* (204) (Figure 6.1). Fibre enriched extracts, namely, elderberry extract (EE), orange extract (OE), and pomegranate extract (PE) were obtained using different extraction procedures, summarized in Figure 6.1.

Portuguese commercial wheat flour type 650 with 14.5 g of moisture, 11 g of protein, 73 g of carbohydrates, 3.5 g of fibre, and 1.6 g of fat per 100 g of flour with a falling number of 220 s was supplied by Forno de Espinho bakery (Espinho, Portugal). Flour improver, fresh yeast, and salt were also supplied by Forno de Espinho bakery (Espinho, Portugal).

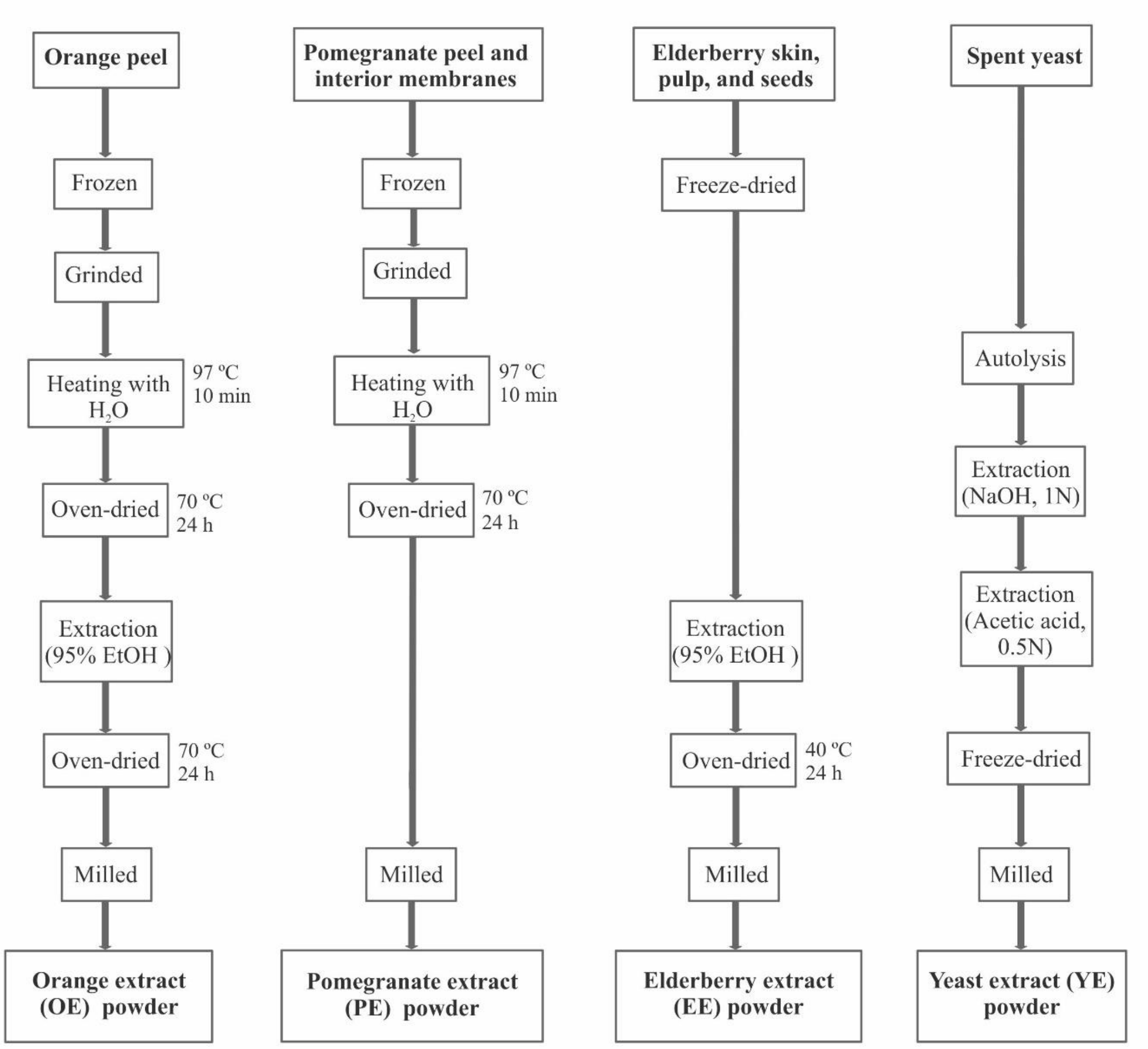

Figure 6.1. Summary of major steps to obtain fibre rich extracts from different agroindustry by-products

6.2.2. Dietary fibre composition

Total dietary fibre (TDF), soluble (SDF) and insoluble dietary fibre (IDF) contents were determined in fibre enriched extracts recovered from BP Additionally, TDF of bread samples was measured. Analyses were performed in triplicate, using commercially available kits (K-TDFR, Megazyme, Cork, Ireland), based on the methods of Prosky *et al.* (228) and Prosky *et al.* (229). Soluble Dietary Fibre (SDF) content was determined as the difference between TDF and IDF values.

6.2.3. Bread samples

Bread recipe was: 400 g wheat flour, 4.0 g flour improver, 8.0 g fresh yeast, 5.2 g salt, and 240.0 g water. Considering the information previously collected from sensory analysis and external preference mapping (results not shown), four different bread formulations were produced with extract addition (% wheat flour): 7.0% EE; 2.5% OE; 5.0%, PE; 2.5% YE. For control, bread without extract addition was prepared. Bread was made using home-making bread machines (Moulinex, Paris, France). The selected bread making program included dough preparation (2:20 h) and baking (20 min). After baking, bread was cooled at room temperature during 90 min before further analysis.

Bread moisture was assayed by infrared drying at 105 °C (Scaltec SMO 01, Heiligenstadt,

Germany) until constant weight and expressed as g of moisture per 100 g of bread.

6.2.4. Microwave-assisted acid digestion

For mineral analysis, bread samples were solubilized through a closed-vessel microwave-assisted acid digestion in a MLS-1200 Mega (Milestone, Sorisole, Italy) microwave oven according to Pinto, Almeida, and Ferreira Pinto *et al.* (299). Briefly, samples (ca. 500 mg) were directly weighted into the polytetrafluoroethylene (PTFE) microwave oven vessels and 3 mL of high-purity concentrated HNO_3 (> 69% w/w, TraceSELECT, Fluka, France) and 1 mL of H_2O_2 (> 30% v/v, TraceSELECT, Fluka, Germany) were added. The mixture was heated using the following microwave oven program (power/time): 250 W/1 min, 0 W/2 min, 250 W/5 min, 400 W/5 min, and 600 W/5 min. After cooling, sample solutions were diluted to 25 mL in decontaminated volumetric flasks with ultrapure water (obtained with an arium® pro ultrapure water system, Sartorius, Goettingen, Germany). Sample blanks were obtained using the same procedure. Each bread type was digested in triplicate.

6.2.5. Simulated gastro-intestinal digestion

Simulated *in vitro* digestion (SIVD) was carried out according to the internationally standardized method described by Minekus *et al.* (300), with the following modification: the intestinal phase was adjusted for non-fat food, eliminating the addition of lipases. The procedure included three consecutive stages: i) oral phase, simulated through an amylase digestion; ii) gastric phase, simulated through a pepsin/HCl digestion; iii) intestinal phase, simulated through a bile salts/pancreatin digestion. Briefly, 5 g of bread sample was mixed with 3.5 ml of simulated salivary fluid (SSF), 0.5 ml of α-amylase solution (1500 U/ml in SSF), 25 μl of 0.3 M $CaCl_2$ solution and 975 μl of water. The mixture (10 ml) was incubated for 2 min and then mixed with 7.5 ml of simulated gastric fluid (SGF), 1.6 ml of pepsin (25,000 U/ml), 5 μl of 0.3 M $CaCl_2$, 200 μl of 1 M HCl and 695 μl of water. The pH was adjusted to 3.0 with 1 M HCl and the water volume added was corrected (695 μl minus the volume of HCl used to reach the required pH). Then, the mixture was incubated in a water bath for 2 h at 37 °C with gentle agitation. The resulting gastric chime (20 ml) was mixed with 11 ml of simulated intestinal fluid (SIF), 5 ml of pancreatin solution (800 U/ml based on trypsin activity), 2.5 ml of bile solution (160 mM), 40 µl of 0.3 M $CaCl_2$, 150 µl of 1 M NaOH and 1.31 ml of water. The pH was adjusted to 7.0 with 1 M NaOH and the water volume added was corrected as aforementioned and mixture was incubated in a water bath for 2 h at 37 ºC. After simulated gastrointestinal digestion, samples were cooled in an ice bath for 10 min, and then centrifuged at 5000 *g* for 10 min at 4 °C, in order to separate the soluble bioaccessible fraction from the residual fraction. Supernatants from the bioaccessible fraction were then filtered through 0.22 µm pore filters and frozen at – 20 °C until further analysis. SSF, SGF and SIF were prepared as described by Minekus *et al.* (300). All digestions were run in triplicates. An additional digestion blank replicate was prepared.

Flame atomic absorption spectrometry (FAAS) and inductively coupled plasma mass spectrometry (ICP-MS) were used to determine the minerals contents in samples. Results were expressed on a fresh weight (fw) basis. The bioaccessible fraction (%) of Ca, Mg, Mn, Fe, Cu, Zn, and Mo in the bread samples was calculated using the equation (I):

$$Bioaccessibility\ (\%) = \frac{Fraction\ of\ the\ element\ in\ the\ in\ vitro\ digested\ sample}{Total\ content\ of\ mineral\ element} \times 100 \tag{6.1}$$

6.2.6. Samples analysis

Ca and Mg determination was performed using an Analyst 200 FAAS instrument (Perkin Elmer, Überlingen, Germany). For Mn, Fe, Cu, Zn, Se and Mo determination, an iCAP Q-ICP-MS instrument (Thermo Fisher Scientific, Bremen, Germany) was used. The following elemental isotopes (m/z ratios) were monitored for analytical determinations: ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{82}Se and ^{98}Mo; the elemental isotopes ^{45}Sc, ^{89}Y, ^{115}In and ^{159}Tb were monitored as internal standards. The limits of detection (LoD) were calculated as the concentration corresponding to 3 times the standard deviation of 10 replicate measurements of the blank solution (2% v/v HNO_3) and are presented in Table 6.1.

Samples solutions from the microwave-assisted acid digestion were simply diluted with ultrapure water while samples from *in vitro* digestion were diluted with a 2% v/v HNO_3 solution and then filtered through 0.45 µm nylon syringe filters before analysis.

Table 6.1. Limits of detection (LoD) for the 8 minerals determined*

Mineral	LoD (µg/100g)
Ca	16.65
Mg	1.55
Cu	0.09
Mn	0.04
Zn	0.21
Fe	4.86
Mo	0.02
Se	0.69

* The ICP-MS instrument was equipped with a MicroMist^{TM} nebulizer (Glass Expansion, Port Melbourne, Australia), standard Peltier-cooled baffled cyclonic spray chamber, quartz torch and two-cone interface design (nickel sample and skimmer cones). High-purity (99.9997%) argon was used as the nebulizer and plasma gas. The ICP-MS instrument was operated under the following conditions: RF power, 1550 W; argon flow rate, 14 L/min; auxiliary argon flow rate, 0.8 L/min; nebulizer flow rate, 1.04 L/min.

6.2.7. Estimated daily intake of minerals

The Estimated Daily Intake (EDI) of major and trace elements resulting from bread consumption was calculated based on the elemental content ($C_{elements}$; mg/100 g bread fw basis) and the average per capita daily consumption of bread (D_{Bread}), using equation (ii):

$$EDI = \frac{C_{elements} \times D_{bread}}{RDA \ or \ AI} \qquad (6.2)$$

where D_{Bread} was assumed as 250 g/person/day (294). EDI was expressed as percentage of RDA (Recommended Dietary Allowance) or AI (Adequate Intake) as recommended by FNB/IOM (301).

6.2.8. Analytical quality control

For analytical quality control purposes, the certified reference material (CRM) BCR 679 (white cabbage, supplied by EC Institute for Reference Materials and Measurements, Geel, Belgium) was analysed for mineral content under the same conditions as the samples. The average recoveries obtained in the CRM analysis are presented in Table 6.2.

Table 6.2. Results obtained from the analysis of the certified reference material BCR 679 (mean ±SD; *n*=3)

Element	Certified value ± uncertainty (µg/g)	Determined value ± standard deviation (µg/g)
Ca	7768 ± 655	7729 ± 186
Mg	1362 ± 127	1338 ± 36
Fe	55.0 ± 2.5	54.4 ± 4.2
Mn	13.3 ± 0.5	13.4 ± 0.5
Cu	2.89 ± 0.12	2.81 ± 0.22
Mo	14.8 ± 0.5	14.7 ± 0.1
Zn	79.7 ± 2.7	79.3 ± 4.0

6.2.9. Statistical analysis

All dependent variables were tested for distribution of the residuals with Shapiro–Wilk's test. The impact of the fortification of bread with fibre rich extracts on the total and bioaccessible mineral content was studied using a one-way analysis of variance (ANOVA), when normal distribution of the residuals was confirmed. Welch correction was applied when homogeneity of variances was not verified. Whenever statistical significances were found, Tukey's or Tamhane's T2 post-hoc tests were applied for mean comparison, depending on, respectively, equal variances assumption or not.

If normal distribution of the residuals was not found, the impact of fibre fortification was studied using a Kruskal–Wallis test. Whenever statistical significances were found, Dunn's post-hoc test was applied for median comparison. Furthermore, the relationship between bread mineral bioaccessibility and TDF was studied using Pearson's correlation.

The analyses were performed at 5% significance level, using XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France).

6.3. Results

6.3.1. Fibre content on agroindustry BP extracts and on bread samples

Results for dietary fibre (DF) fractions extracted from agroindustry BP are presented in Figure 6.2. Significant differences ($p < 0.05$) were found between extracts for Total dietary fibre (TDF), Insoluble dietary fibre (IDF), and Soluble dietary fibre (SDF). TDF values ranged between 40.9 (Elderberry extract, EE) and 72.9 g/100 g (Orange extract, OE). Insoluble dietary fibre (IDF) values were between 29.3 (EE) and 70.8 g/100 g (OE), representing more than 70% of the DF present in the extracts. Soluble dietary fibre (SDF) was the minor DF fraction, and varied from 2.1 (OE) to 20.1 g/100 g (Yeast extract, YE).

For TDF, the contribution of the extracts was (per 100 g of bread, fw): 2.14 g (EE); 1.59 g (OE); 2.69 g (PE); 1.69 g (YE). Regarding IDF content, the extracts contributed with (per 100 g bread, fw): 1.53 g (EE); 1.54 g (OE); 2.36 g (PE); 1.22 g (YE). Lastly, the contribution of the extracts for the amount of SDF was (per 100 g bread, fw): 0.61 g (EE); 0.05 g (OE); 0.34 g (PE); 0.47 g (YE).

Concerning TDF content of bread samples, control bread contained 3.37 g/100g fresh weight (fw), as expected, bread formulations fortified with fibre enriched extracts presented higher TDF content: OE, 4.83 g/100g (fw); EE, 5.32 g/100g (fw); PE, 6.14 g/100g (fw); and YE, 5.10 g/100g (fw).

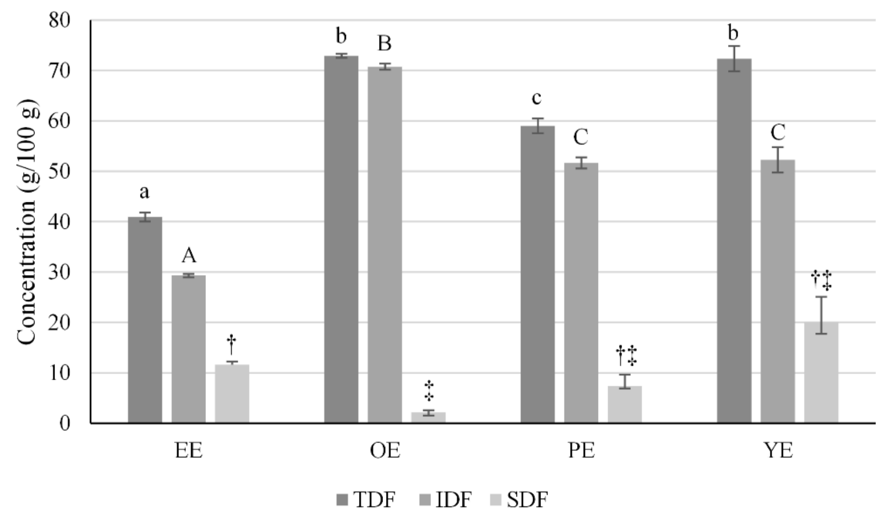

Figure 6.2. Dietary fibre composition of extracts (g/100 g), on dry weight basis.
Data expressed as mean ± standard deviation (n = 3).
EE, Elderberry extract; IDF, Insoluble dietary fibre; OE, Orange extract; PE, Pomegranate extract; SDF, Soluble dietary fibre; TDF, Total dietary fibre; YE, Yeast extract.
Values with the same letter or symbol (lower-case letters for TDF, upper-case letters for IDF, and symbols for SDF) do not differ significantly ($p < 0.05$) from the given mean.

6.3.2. Total and bioaccessible mineral content of fortified wheat bread

Average values for moisture content in the different wheat bread formulations varied from 35.8% to 38.0%, with no significant differences between them ($p > 0.05$). Total mineral content of control bread and wheat breads fortified with fibre rich extracts are shown in Table 6.3. The elements content in each bread formulation was in the following order: Na > K > Ca > Mg > Fe > Mn > Zn > Cu > Mo > Se. Significant differences ($p < 0.05$) were found between breads and, in general, lower values were found for control bread.

Bioaccessible minerals content in control and wheat bread fortified with fibre rich extracts is shown in Table 6.3. The order of elements content in control, EE, PE, and YE breads was as follows: Ca > Mg > Fe > Zn> Mn > Cu > Mo. For OE bread, the order was: Ca > Mg > Fe > Mn > Zn > Cu > Mo. The amount of bioaccessible mineral differed significantly between bread formulations.

Table 6.3. Total and bioaccessible mineral content of wheat bread fortified with different extracts (% wheat flour), on a fresh weight basis.

Element	**Concentration**	**Extract (% wheat flour)**					***p***
		C (0.0)	EE (7.0)	OE (2.5)	PE (5.0)	YE (2.5)	
Na	Total[1]	348.2 ± 13.5^{ab}	342.6 ± 15.8^{a}	341.1 ± 0.7^{a}	369.0 ± 12.7^{b}	354.6 ± 12.5^{ab}	0.009*
	Bioaccessible[1]	na	na		na	na	-
K	Total[1]	127.4 ± 4.6^{a}	175.1 ± 7.0^{b}	126.8 ± 5.1^{a}	171.1 ± 5.4^{b}	131.8 ± 6.3^{a}	<0.001*
	Bioaccessible[1]	na	na		na	na	-
Ca	Total[1]	119.6 ± 4.9^{a}	120.6 ± 5.3^{a}	129.7 ± 4.7^{b}	131.9 ± 1.9^{b}	120.4 ± 6.4^{a}	<0.001*
	Bioaccessible[1]	1.8 ± 0.2^{a}	2.7 ± 0.4^{b}	3.9 ± 0.5^{c}	2.1 ± 0.2^{ab}	7.9 ± 0.5^{d}	<0.001**
Mg	Total[1]	27.3 ± 0.6^{a}	36.1 ± 1.6^{b}	28.1 ± 0.8^{ac}	29.2 ± 0.9^{c}	28.8 ± 0.6^{ac}	<0.001*
	Bioaccessible[1]	2.7 ± 0.1^{a}	4.1 ± 0.4^{b}	3.0 ± 0.2^{c}	2.4 ± 0.2^{d}	8.4 ± 0.4^{e}	<0.001**
Mn	Total[2]	683.8 ± 20.0^{a}	823.4 ± 28.5^{b}	694.4 ± 25.7^{ac}	750.3 ± 3.9^{d}	735.1 ± 30.8^{cd}	<0.001*
	Bioaccessible[2]	53.3 ± 4.4^{a}	131.8 ± 9.9^{b}	120.0 ± 0.4^{c}	11.1 ± 0.5^{d}	169.6 ± 9.7^{b}	<0.001**
Fe	Total[2]	1517.1 ± 50.0^{a}	1728.0 ± 8.1^{b}	1724.7 ± 72.9^{b}	1868.8 ± 81.6^{c}	1578.6 ± 27.9^{a}	<0.001**
	Bioaccessible[2]	119.8 ± 6.3^{a}	251.3 ± 13.0^{b}	187.2 ± 12.5^{c}	63.0 ± 4.1^{d}	395.9 ± 24.7^{e}	<0.001**
Cu	Total[2]	90.1^{a} (86.4-93.5)	117.3^{bc} (116.4-118.2)	126.9^{b} (122.3-136.8)	115.6^{abc} (115.0-115.7)	105.9^{ac} (101.5-110.7)	<0.001***
	Bioaccessible[2]	33.5 ± 1.1^{a}	36.1 ± 2.4^{ab}	34.2 ± 2.2^{a}	9.2 ± 0.5^{c}	39.3 ± 2.3^{b}	<0.001*
Zn	Total[2]	623.0^{a} (609.8-646.4)	629.4^{a} (624.5-702.3)	637.7^{a} (590.8-684.8)	837.1^{b} (797.5-883.3)	697.6^{ab} (659.1-755.4)	<0.001***
	Bioaccessible[2]	88.1 ± 5.6^{a}	119.7 ± 16.6^{b}	93.2 ± 8.7^{a}	28.8 ± 2.7^{c}	219.1 ± 14.7^{d}	<0.001**
Se	Total[2]	2.9^{a} (2.5-3.9)	3.6^{ab} (3.4-3.7)	3.3^{ab} (2.4-3.7)	5.2^{b} (4.7-6.2)	2.6^{a} (2.1-2.9)	<0.001***
	Bioaccessible[2]	na	na	na	na	na	-
Mo	Total[2]	12.9 ± 0.5^{a}	14.5 ± 0.6^{b}	13.6 ± 0.7^{ab}	17.9 ± 0.5^{c}	14.4 ± 0.7^{b}	<0.001*
	Bioaccessible[2]	7.2 ± 0.3^{a}	1.4 ± 0.1^{b}	5.9 ± 0.4^{c}	BLD	10.5 ± 0.3^{d}	<0.001*

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n = 6).

BLD, below limit of detection (0.02 µg/ 100g for Mo); C, Control bread; EE, Elderberry extract; OE, Orange extract; na, not available; PE, Pomegranate extract; YE, Yeast extract.

[1] (mg/100 g)

[2] (µg/100 g)

Different letters for each element in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* p Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** p Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** p Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test.

6.3.3. Mineral bioaccessibility and relationship with dietary fibre fractions

As shown in Figure 6.3, bioaccessibility varied significantly between wheat bread formulations. YE bread showed the highest values for Ca, Mg, Mn, Fe, Zn, and Mo (6.6 ± 0.7%, 29.4 ± 1.7%, 23.1 ± 1.9%, 25.7 ± 2.8%, 31.1 ± 1.6%, and 72.9 ± 4.3%, respectively). Control bread presented the highest values for Cu (37.3 ± 2.0%) and lowest for Ca (1.5 ± 0.2%). The lowest bioaccessibility for Mg, Mn, Fe, Cu, and Zn was found in PE bread (8.2 ± 0.6%, 1.5±0.1%, 3.4 ± 0.2%, 7.9 ± 0.4%, and 3.4 ± 0.3%, respectively). For Mo, the lowest bioaccessibility was observed in PE bread. Regarding this element, the bioaccessible fraction was below the LoD (0.02 µg/100 g), therefore, its bioaccessibility was not reported.

The relationship between the mineral bioaccessibility and the TDF content in all bread types was examined. A significant negative correlation was found between TDF and Cu (r_p = -0.734), and also between TDF and Mo (r_p = -0.644). For other elements, the correlations were not statistically significant.

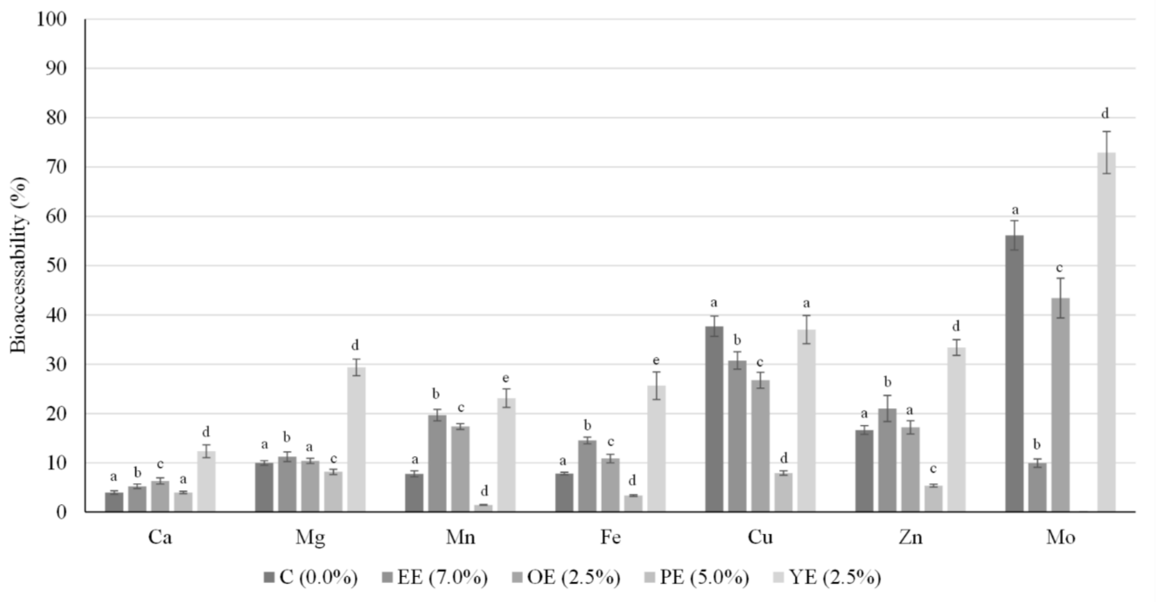

Figure 6.3. Bioaccessibility (%) of Ca, Mg, Mn, Fe, Cu, Zn, and Mo in fortified breads.
Data expressed as mean ± standard deviation or as median (minimum−maximum), (n = 6). C, Control bread; EE, Elderberry extract; OE, Orange extract; PE, Pomegranate extract; YE, Yeast extract. Values with the same letter in the same element do not differ significantly (p < 0.05) from the given mean.

6.4. Discussion

TDF content in extracts from agroindustry BP was in agreement with literature, except for EE, which presented a lower content (23, 235-240). Regarding to IDF fraction, the values found were higher than those reported (86% and 73%, respectively) for PE and YE (237-

240). Higher proportion of IDF compared to SDF may result from the use of water in the extraction procedures, which reduces SDF content.

Taking into account the TDF content in bread samples, all the studied formulations could be eligible for the nutrition claim “source of fibre” (≥ 3 g TDF/100 g bread, fresh weight) (13). EE fortified bread showed the highest content of K, Mg, and Mn (37%, 32%, and 20% higher than control bread, respectively), while OE fortified bread presented the highest Cu content (41% higher than control bread). PE fortified bread presented the highest values of Na, Ca, Fe, Zn, Se, and Mo (6%, 10%, 23%, 34%, 83%, and 38% higher than control bread, respectively). Additionally, significant differences ($p < 0.05$) were found between control bread and: EE and PE breads, for K and Mg; OE and PE breads, for Ca; EE, PE, and YE breads, for Mn; EE, OE, and PE breads, for Fe; EE and OE breads, for Cu; PE bread, for Zn and Se; EE, PE, and YE breads, for Mo.

The mineral content of wheat bread formulations was compared to the content described in several Food Databases (225-227, 302-308). The values found for control and YE fortified bread were within the ranges reported (with no significant differences, $p > 0.05$) for all elements, except Mn. For this element, all bread formulations presented significantly higher content than that reported in Food Databases (Control: + 41%, $p = 0.007$; YE: + 51%, $p = 0.001$; EE: + 69%, $p < 0.001$; OE: + 43%, $p = 0.004$; PE: + 54%, $p = 0.001$). Additionally, the incorporation of EE to wheat bread increased Mg (+ 39%, $p = 0.006$) and K (+ 44%, $p < 0.001$), bread fortification with PE increased K (+ 43%, $p < 0.001$); Ca (+ 158%, $p < 0.001$), and Fe (+ 23%, $p = 0.001$), whereas OE supplementation increased Ca content (+ 112%, $p = 0.002$). The comparison with Food Databases indicates that fortification with fibre rich extracts can also improve the content of some essential minerals in wheat bread.

Estimation of the Daily Intake (EDI) of essential elements as % of RDA (Recommended Dietary Allowances) or AI (Adequate Intakes) supposing an average *per capita* consumption of 250 g of each bread type per person per day is shown in Table 6.4. The EDI was calculated for adults aged 19–50 years, taking into account the WHO’s recommendation for bread daily intake. Considering major elements, all bread formulations would mainly contribute to daily requirements of Na (from 58.0 to 61.5%). For Ca, control bread would fulfil 29.9% of the recommendations, whereas OE and PE breads would contribute to 32.4% and 33.0%, respectively. While control bread would fulfil 16.3% or 21.3% of the daily requirements of Mg, EE fortified bread contribution would be higher (21.5% and 28.2%). Regarding to the contribution for dietary requirements of trace elements, differences were found between control bread and fibre enriched bread formulations. EE bread presented the highest contribution to Mn daily requirements (Table 6.4). Besides EE bread, PE and YE breads would also cover requirements of Mn in females (Table 6.4). PE bread provided the highest contribution to Fe, Zn, Se, and Mo daily requirements (26.0 and 58.4%, 19.0

and 26.2%, 23.6 and 99.4%, respectively). OE bread presented the highest contribution of Cu (35.3%). Overall, the contribution of bread formulations fortified with fibre rich extracts to minerals requirements, especially PE fortified bread, is relevant. Moreover, it is noteworthy that, compared to the control bread, YE bread contribution to minerals requirements was not as noticeable as with bread fortified with other fibre rich extracts.

Table 6.4. Estimated daily intake (EDI) of essential elements considering the average *per capita* consumption of bread (250 g/person/day).

	EDI (expressed as % of the RDA or **AI**)										RDA or AI (mg/day)	
Element	C (0.0)		EE (7.0)		OE (2.5)		PE (5.0)		YE (2.5)			
	F	M	F	M	F	M	F	M	F	M	F	M
Na	58.0		57.1		56.9		61.5		59.1		1500	
K	6.8		9.3		6.7		9.1		7.0		4700	
Ca	29.9		30.2		32.4		33.0		30.1		**1000**	
Mg	21.3	16.3	28.2	21.5	22.0	16.7	22.8	17.4	22.5	17.1	320	420
Mn	95.0	74.3	114.4	89.5	96.4	75.5	104.2	81.6	102.1	79.9	**1.8**	**2.3**
Fe	21.1	47.4	24.0	54.0	24.0	53.9	26.0	58.4	21.9	49.3	18	8
Cu	25.0		32.6		35.3		32.1		29.4		0.9	
Zn	19.5	14.2	19.7	14.3	19.9	14.5	26.2	19.0	21.8	15.9	8	11
Se	13.2		16.4		15.0		23.6		11.8		0.055	
Mo	71.7		80.6		75.6		99.4		80.0		0.045	

RDAs (Recommended Dietary Allowances) and AIs (Adequate Intakes) may both be used as goals for individual intake. RDAs are in regular type and AIs in bold type. Underlined values were calculated from values taken by *in vitro* intestinal bioaccessibility results.
C, Control; EE, Elderberry extract; F, Female; OE, Orange extract; M, Male; nd., not detected (below detection limit); PE, Pomegranate extract; YE, Yeast extract.

Although promising results were obtained for total mineral content (and, therefore, to EDI), the eventual presence of anti-nutrients could influence minerals bioaccessibility. As expected, bioaccessible mineral levels decreased. This reduction varied at different extents, depending on the wheat bread formulation. Compared to the control bread, lower values of bioaccessible fraction were found in PE fortified bread for Mg (-12%), Mn (-79%), Fe (-48%), Cu (-73%), Zn (-67%), and Mo (values lower than LoD). YE fortified bread showed the highest bioaccessible mineral levels: Ca (339%), Mg (211%), Mn (218%), Fe (230%), Cu (17%), Zn (149%), and Mo (45%) *versus* control bread. Moreover, significant differences were found between control bread and: EE, PE and YE breads, for Ca and Mo; EE, OE, PE, and YE breads, for Mg, Mn, and Fe; PE and YE breads, for Cu; EE, OE, and YE breads, for Zn.

Contrary to what was observed for total mineral content, bioaccessibility in YE bread was significantly higher than in the other bread formulations, while the opposite was observed for PE bread. These changes may be due to the fibre rich extracts composition, specifically the presence of anti-nutrients. In a study by Barros *et al.* (309), tannins and phytic acid were detected in pomegranate peels in considerable amounts (38.25 ± 2.87 mg catechin equivalent/g and 14.71 ± 0.73 mg phytic acid equivalent/g, respectively; dry weight basis). Tannins were also quantified in orange peel, ranging from 3.87 ± 0.09 to 6.43±0.06 mg catechin equivalent/g (dry weight basis) for proanthocyanidins and 4.48 ± 0.24 to 16.28 ± 0.36 mg tannic acid equivalents/g (dry weight basis) for hydrolysable tannins (310). Okpala and Akpu (311) determined tannins content in bread with different concentrations of orange peel flour, and found that orange peel flour significantly increased tannin content, which ranged from 6.7 ± 1.5 to 8.0 ± 1.3 mg/g. Moreover, oxalic acid was detected and quantified by Ersus and Cam (312) at 257.5±25.3 mg/100 g and by Clements (313) ranging from 0.05 to 0.2 Meq/g (dry weight basis). Regarding elderberry BP, no studies on anti-nutrient content are available. However, proanthocyanidins were reported in elderberry at 23.3 mg/100 g fruit (262). While a negative influence on minerals bioaccessibility would be expected due to anti-nutrients presence in the fibre rich extracts, this effect was only evident for PE. This may be due to the presence of higher amounts of anti-nutrients in this extract, which would result from the extraction procedures applied: water at 97 ºC followed by 95% ethanol extraction, for OE; 95 % ethanol extraction, for EE; water at 97 ºC, for PE. Heating temperature and extraction time used were lower than that usually reported for reducing anti-nutrients in legumes (100 ºC, 35 to 90 min) (314, 315). Additionally, ethanol extraction is more effective for anti-nutrient reduction than the heating step (313, 316, 317). Concerning brewer’s spent yeast, no reports on oxalic acid, phytic acid, or tannins content are available. However, according to the literature, the addition of brewer’s spent yeast autolysate can favour the microorganisms growth during fermentation (318) and yeasts have the ability to synthesize and release phytases during the fermentation process (319, 320). Therefore, higher proliferation of baker’s yeast during dough fermentation could lead to an increase in phytase activity and consequently reduced phytate content in wheat bread fortified with YE, thus less chelation effect on minerals would occur.

Scarce correlation was found between TDF and minerals bioaccessibility, strong negative correlation was only found between bread TDF content and Cu and Mo bioaccessibility. Therefore, other anti-nutrients (oxalic acid, phytic acid, and tannins), usually associated with DF, could influence the observed mineral bioaccessibility (321).

6.4. Conclusions

Wheat bread fortification with OE, EE, and PE improved the content of essential minerals when compared with the control bread. Thus, fortification of bread formulations with fibre rich extracts, especially PE extract, seems a promising strategy to meet the daily mineral requirements. The exception was fortification with YE, since fortified bread presented a mineral content similar to control bread (with no fortification). However, bioaccessibility assays highlighted that, unlike what was observed for total mineral content, minerals bioaccessibility in YE fortified bread was significantly higher compared to the other bread fortifications (OE, EE, and PE). The opposite, i.e., a significant decrease in minerals bioaccessibility was observed for PE.

Bread fortification with fibre rich extracts obtained from agroindustry BP significantly changes its mineral composition. However, the nature of the fibre rich extract must be carefully selected and studied, because a strong negative impact can also occur on minerals bioavailability.

PART V

CHAPTER 7

Conclusions and future prospects

Functional ingredients obtained from industrial by-products (BP) are promising for improvement of nutritional quality of bread and may potentially enhance their health promoting properties. However, the incorporation of BP functional ingredients also influence technological and sensorial properties.

The preliminary study performed in this thesis using brewer's yeast showed that dry spent yeast contributed to an increase (1.9 times) of β-glucans content in fortified breads. Fortification with dry spent yeast had impact on bread colour, texture and volatile compounds, since it resulted in darker crumb, increased crumb and crust springiness, and increased hexanal content. However, it had no major impact on sensory acceptability. Dry spent yeast presented promising results for brewer's yeast reuse and as a source of β-glucans, but required some improvement in order to enhance β-glucans content. Hence, β-glucans extraction from brewer's spent yeast was optimized. In the next stage of this preliminary study, the impact of bread fortification with β-glucans rich extract and protein/proteolytic enzymes extract on physical characteristics was evaluated. Compared with control bread, addition of β-glucans rich extract improved bread quality while proteins/proteolytic enzymes extract had a negative impact. Therefore, proteins/proteolytic enzymes extract was not considered for futher studies.

Together with β-glucans (fibre) rich extract obtained from brewer's spent yeast (YE), three agroindustry BP of plant origin were also used to obtain fibre rich extracts: elderberry skin, pulp and seeds (for elderberry extract, EE); orange peel (for orange extract, OE); pomegranate peel and interior membranes (for pomegranate extract, PE). Extraction procedures were optimized for each BP, according to their characteristics, aiming to obtain fibre rich extracts, which were then characterized on their fibre composition. The highest total dietary fibre (TDF) content was found for OE and YE, while the lowest was for EE. Also, insoluble dietary fibre (IDF) fraction represented 66.48 to 98.41% of TDF.

Functional breads presenting the fibre related nutrition claims "source of fibre" and "high in fibre" were then prepared through fortification with YE (4%), EE (4 and 36%), OE (4 and 8%), and PE (4 and 16%). Different concentrations were selected for statistical purposes concerning the evaluation of dietary fibre (DF) impact on studied parameters, regardless of extract used (more than three DF concentration points were required to obtain reliable models in multivariate partial least squares (PLS) regression analyses). Firstly, dough characteristics were analysed, including mechanical properties, microstructure and fermentation. Compared to the control, optimum water absorption (OWA) increased for doughs fortificatied with OE, PE, and YE, while an opposite tendency was found for EE. Also, EE, PE, and YE fortification resulted in a shortened dough development time (DDT), whereas there was an increase for OE. Values for shear modulus at the onset of starch gelatinization ($|G^*|_{onset}$) were significanlty higher than control for 16% PE and 4% EE. For maximum shear modulus ($|G^*|_{max}$), differences were significant for 8% OE, 4% EE, and 36% EE. Moreover, average temperature values at |G*|onset and |G*|max were not statistically different from the control dough. Regarding maximum loss factor (tan δ_{max}), values significantly decreased compared with the control, except for 4% EE and 4% OE. As for temperature, an early arrival to tan δ_{max} was registered, except for 36% EE. Confocal laser scanning microscopy (CLSM) analysis indicated structural changes at varying extents, depending on extract and replacement level, and were in accordance with results from fundamental rheology. As expected, in the control dough the protein network was well formed and some empty areas between protein strands could be detected. Compared with the control dough, protein newtork of fortified doughs was: i) more spread with 4% YE; ii) more spread and distributed, but a bit weakened in dough containing 4% OE; iii) more clustered and stretched with increase in OE to 8%; iv) slightly inhibited, with proteins with some irregularity in distribution, and partially agglomerated for 4% PE; v) not visible with 16% PE; vi) less interconnected with 4% EE; vii) less interconnected and somewhat inhibited, with agglomerated proteins for 36% EE. As regards to fermentation characteristics, when compared with the control dough, maximum dough height (H_m) significantly decreased in all fortified doughs. The same decrease tendency was observed for final dough height (h), except for 4% EE. The time to reach maximum dough development (T1) significantly increased, compared to the control, for 36% EE, 4% PE, 16% PE, and 4% YE, whereas the opposite was observed for 4% OE. Dough fortification with 36% EE and 4% PE significantly decreased maximum height of gaseous production (H'_m) and increased the time of maximum gas formation (T’1). Statistically, wheat flour fortification had more impact on dough development than on gas production throughout fermentation, being in accordance with what was observed in rheology and microstructure analyses. Bread characteristics, including volume, texture, and image analysis were analysed after completing dough analysis. Bread volume and specific volume significantly decrease for 36% EE, 8% OE, 4% PE, and 16% PE, when compared to the control bread. Yet, bread volume for 4% EE, 4% OE and 4% YE significantly increased. Extract fortification also altered breads colour and height. Crumb colour was darker, while height increased with 4% EE and decreased for PE, 36% EE, and 8% OE. In relation to bread cell attributes the total number of cells did not present major changes from the control, apart from 4% YE and 36%. A significant increase in cells with area > 5.0 mm^2 (large cells) was detected for 36% EE, 4% OE, 16% OE, and 4% YE which could be explained by dough H_m and h results. The crumb hardness was significantly higher than control for 8% OE, 4% PE, and 16% PE, and lower for 4% EE. Bread fortification with 36% EE, 4% PE, and 8% PE altered significanlty the cohesiveness. Chewiness decreased with EE and increased with 8% OE and 16% PE, compared with the control bread. Dough and bread analysis revealed the impact of fortification with EE, OE, PE, and YE. This influence could be related with TDF, IDF, and/or SDF contribution of each extract. Multivariate PLS regression was performed in order to study the correlation of bread physical parameters, crumb texture, and crumb image analysis data with dough empirical and fundamental rheology, based on bread characteristics data prediction from dough parameters data. Results obtained from this analysis highlighted the impact of DF fortification on dough properties, which was proportional to the extract amount added. Moreover, TDF content appears to have a stronger influence than its profile (SDF and IDF fractions). Overall, the impact of wheat flour replacement on dough and bread characteristics varied with extract origin and replacement levels. Extract fortification at levels suitable for “high in fibre” claim (8% OE, 16% PE, and 36% EE) had a notorious negative impact on dough and bread properties, while fortification levels suitable for “source of fibre” claim (4%) had less impact. Therefore, although “high in fibre” bread was not viable, it was possible to successfully obtain breads with the nutrition claim “source of fibre”.

Despite their nutritional/health benefits, breads formulated with new functional ingredients must have high sensory acceptance in order to be selected and eaten. Otherwise, they will not be able to compete with traditional wheat bread. External preference mapping was suitable to study consumer preferences and to select the concentrations of fibre rich extracts with best acceptance i.e., 7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE. At these concentrations, all bread formulations were elegible for the nutrition claim “source of fibre”. Regarding colour and crumb structure, bread formulations with 7.0% EE and 5.0% PE registered more differences than 2.5% OE and 2.5% YE, compared to control bread. Additionally, multivariate PLS regression provided information on the relationship between sensory and instrumental data (colour and crumb structure). Therefore, mathematical modelling was shown as a relevant tool not only to study bread acceptability but also to understand relationships between sensory and instrumental data.

Results obtained for bread fortified with fibre rich extracts (7.0% EE, 2.5% OE, 5.0% PE, and 2.5% YE) were promising, but it was also important to evaluate the impact on other nutrients, such as minerals. Wheat bread fortification with 2.5% OE, 7.0% EE, and 5.0% PE improved the content of essential minerals when compared with the control bread. Thus, fortification of bread formulations with fibre rich extracts, especially PE extract, seems a promising strategy to meet the daily mineral requirements. The exception was fortification with 2.5% YE, since this bread presented a mineral content similar to control bread (with no fortification). However, bioaccessibility assays highlighted that, unlike what was observed for total mineral content, minerals bioaccessibility in 2.5% YE fortified bread was significantly higher compared to the other bread fortifications (2.5% OE, 7.0% EE, and 5.0%

PE). The opposite i.e., a significant decrease in minerals bioaccessibility was observed for PE.

Overall, the work developed and presented in this thesis demonstrates the potential of agroindustry by-products as a way to improve bread nutritional/health properties, without major impact on dough and bread characteristics, and with sensory acceptability. Moreover, fibre rich extract obtained from brewer's spent yeast appears to have greater potential to be used in bread production. This reuse of agroindustry BP is in alignment with European Commission Directive 2008/98/EC on waste (Waste Framework Directive), which sets the basic concepts and definitions related to waste management and lays down waste management principles.

The assays performed in this thesis provide relevant information not only at laboratorial scale, but in some cases at pilot scale. However, implementation at industrial scale will bring some additional challenges, since it would be necessary to guarantee: i) microbiological BP safety; ii) industrial efficiency of extraction procedures; iii) assure extract safety and quality at large scale. It is also possible to identify some challenges and opportunities regarding viability of implementation on food industry sector. Although reuse of agroindustry BP supports waste reduction and contributes to indirect income generation, it will require an initial capital investment for the implementation at industrial scale. Also, as BP producers usually are not nearby food transformation industry facilities there will be an additional cost for transportation. Therefore, these and other difficulties must be overcome in order to make the transformation of a waste into an ingredient for the food industry an appealing venture.

PART VI

CHAPTER 8

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